



US007402732B2

(12) **United States Patent**
Ito et al.

(10) **Patent No.:** **US 7,402,732 B2**
(45) **Date of Patent:** **Jul. 22, 2008**

(54) **PARAQUAT RESISTANCE GENE AND A VASCULAR TISSUE—AND TRICHOME-SPECIFIC PROMOTER**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 300 days.

(21) Appl. No.: **10/937,710**

(22) Filed: **Sep. 10, 2004**

(65) **Prior Publication Data**

US 2005/0091711 A1 Apr. 28, 2005

(30) **Foreign Application Priority Data**

Sep. 12, 2003 (JP) 2003-322051

(51) **Int. Cl.**
C12H 15/82 (2006.01)

(52) **U.S. Cl.** **800/278**

(58) **Field of Classification Search** None
See application file for complete search history.

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(57) **ABSTRACT**

A paraquat resistance gene and a vascular tissue- and trichome-specific promoter are provided. The paraquat resistance gene and the vascular tissue- and trichome-specific promoter are isolated by identifying and analyzing genes of *Arabidopsis thaliana*.

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FIG. 1

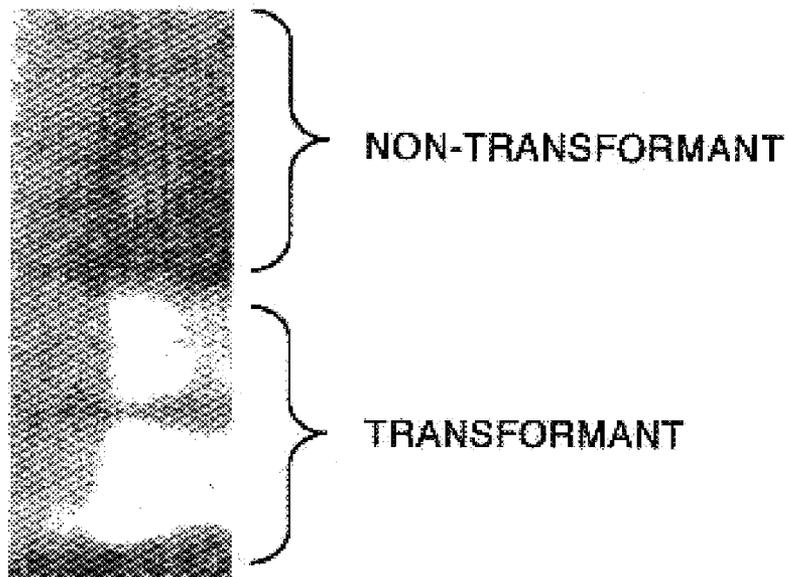
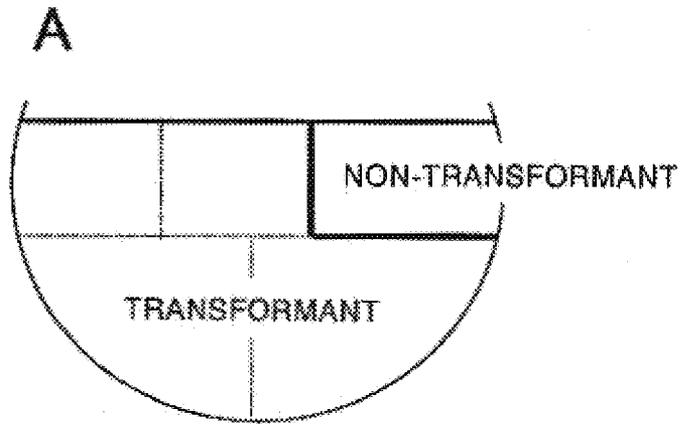
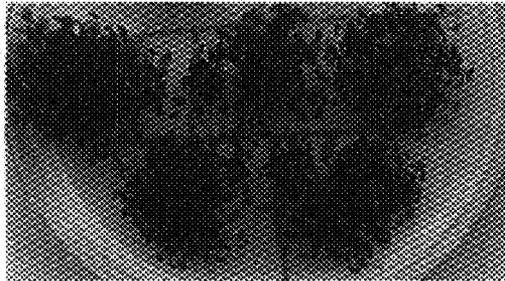


FIG. 2



B



C

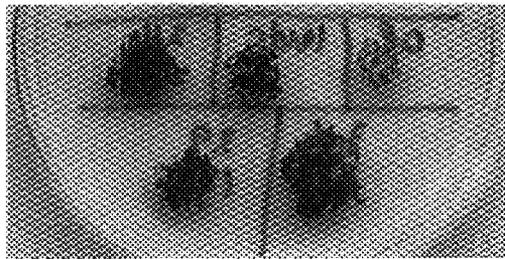


FIG. 3

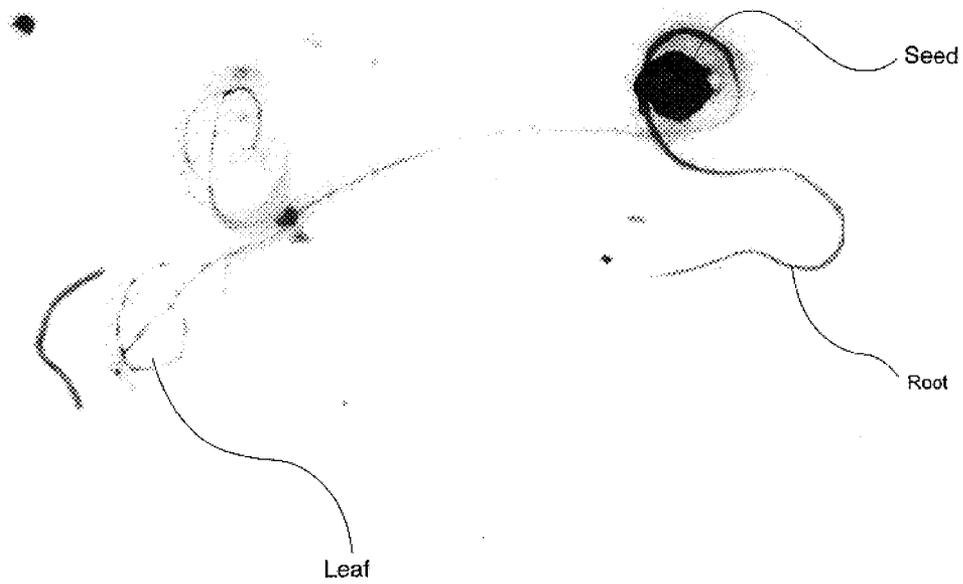


FIG. 4

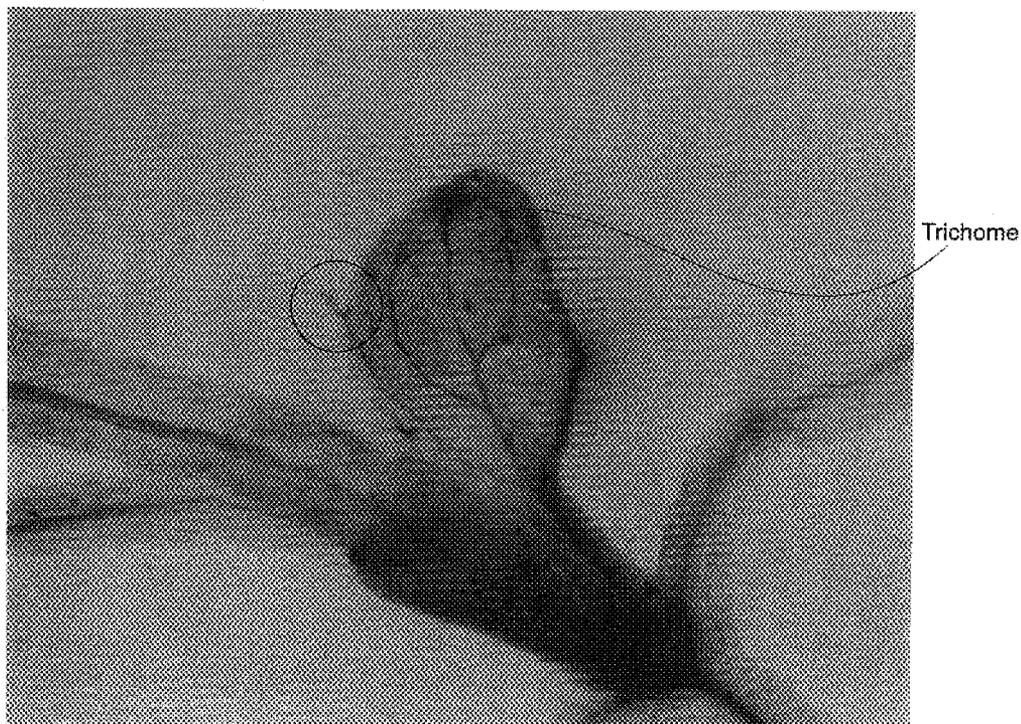
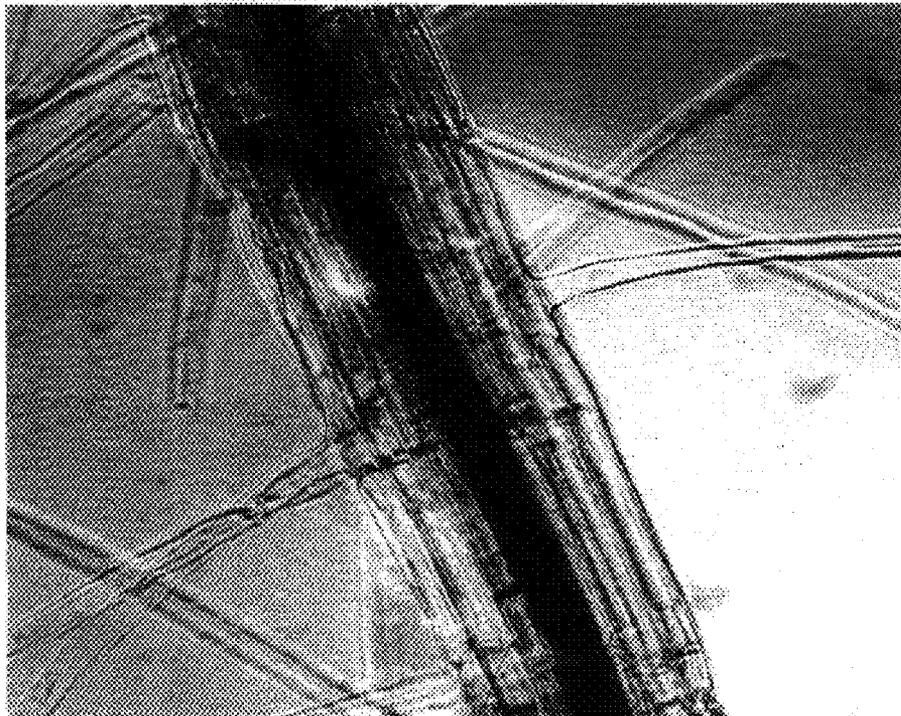


FIG. 5



Vascular tissue

**PARAQUAT RESISTANCE GENE AND A
VASCULAR TISSUE—AND
TRICHOME-SPECIFIC PROMOTER**

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a paraquat resistance gene imparting paraquat resistance and a vascular tissue- and trichome-specific promoter.

2. Background Art

Plants are exposed to various environmental stresses on a regular basis including high and low temperatures, drought, high light intensity, salinity, air pollutant gases, pathogenic microbes and the like. Therefore, if useful plants that can grow sufficiently even under such types of environmental stresses such as, for example, crops, can be developed, food production will become possible even in regions in which crops and the like can not currently grow due to environmental stresses, and the possibility of being prepared for a grave food crisis that is forecast in the future will be increased. Consequently, the production of plants that have improved resistance to such kinds of environmental stresses is underway on a global basis. For example, plants have been produced that were imparted with chilling resistance (Nature, 356, 710-703, 1992; Plant Physiol., 105, 601-605, 1994), drought resistance (Plant Physiol., 107, 125-130, 1995; Nature, 379, 683-684, 1996; Nature Biotech., 17, 287-291, 1999), salt resistance (Science, 259, 508-510, 1993; Biotechnology, 14, 177-180, 1996; Plant J., 12, 133-142, 1997), air pollutants resistance (Plant Cell Physiol., 34, 129-135, 1993; Biotechnology, 12, 165-168, 1994), disease resistance (Kagaku to Seibutsu (Chemistry and Organisms), 37, 295-305, 385-392, 1999) and the like by genetic recombination techniques. Further, some plants that have been imparted with resistance to agricultural chemicals by genetic recombination techniques are in practical use (Nature, 317, 741-744, 1985; Proc. Natl. Acad. Sci. USA, 85, 391-395, 1988; EMBO J., 6, 2513-2518, 1987; EMBO J., 7, 1241-1248, 1988).

These environmental stresses are closely related with in vivo generation of active oxygen species (superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxy radical (OH^\cdot)). Active oxygen species are generated by respiration, photosynthesis, environmental stresses and the like, and impart fatal damage to cells by excessive oxidation of proteins, nucleic acids, membrane structure or the like. It has also been reported that an active oxygen-resistant plant produced by genetic recombination techniques showed improved resistance to the aforementioned environmental stresses (Plant Physiol., 111, 1177-1181, 1996; FEBS Letters, 428, 47-51, 1998).

To produce an active oxygen-resistant plant, a method is principally employed in which a gene of enzyme scavenging active oxygen species (superoxide dismutase, ascorbate peroxidase, catalase and glutathione reductase and the like) is introduced into the plant.

Paraquat is a non-selective and potent herbicide that can kill all plants by continuously generating active oxygens in the photosystems. Paraquat resistance can thus be used as an indicator of the resistance to active oxygens, and analysis concerning the mechanism of paraquat resistance in plants has been conducted (Pestic. Biochem. Physiol., 26, 22-28, 1986; Theor. Appl. Genet. 75, 850-856, 1988; and Plant Physiol., 91, 1174-1178, 1989).

Meanwhile, an apoptosis suppressor gene (JP Patent Publications (Kokai) No. 10-309142; No. 2000-23583; and No. 2002-300822), a gene encoding a protein homologous to

aldose reductase (JP Patent Publication (Kohyo) No. 2001-523466) and a gene encoding an iron-binding protein (ferritin) (JP Patent Publication (Kohyo) No. 2001-519671) have been disclosed as genes that can impart paraquat resistance. Further, in JP Patent Publications (Kokai) No. 2002-281979 and No. 2001-95585, peroxidase derived from paraquat resistant callus is disclosed as a gene capable of imparting resistance to paraquat.

It had been believed that if a paraquat resistance gene that can impart strong resistance to paraquat could be isolated, it would be useful in the development of plants with high resistance to active oxygens generated under various kinds of environmental stress conditions (high and low temperatures, drought, high light intensity, salinity, air pollutant gases, pathogenic microbes and the like). However, recently it has been revealed that active oxygens fulfill an important role as a molecule regulating the growth and stress response of a plant. Therefore, to avoid influencing important characteristics such as crop yield, it is important to increase the resistance of a plant to stresses such as paraquat without affecting the growth and physiological control mechanisms of a plant dependent on active oxygens.

The vascular tissue is a fascicular tissue system that differentiates through each organ of pteridophytes and spermatophytes, such as the stem, leaf and root. Xylem and phloem are the components of the vascular tissue, and they function as pipes to transport water and internal substances throughout the plant. Further, the vascular cambium, which includes the interfascicular cambium and the intrafascicular cambium, is found in the vascular tissue. The vascular cambium is a site of cell proliferation, and is thus an extremely important site for the growth of a plant. Thus, the vascular tissue is a location involved in transporting water and internal substances as well as cell proliferation in a plant. Accordingly, if a gene involved in transporting water or internal substances or in cell proliferation can be introduced into a plant and expressed specifically in the vascular tissue, it will be possible to regulate the transport of water or internal substances or cell proliferation in the plant.

In addition, from the viewpoint of plant diseases, the vascular tissue is a site where a wilt disease fungus infecting plants of the family Solanaceae proliferates and transfers. When a plant virus infects a plant, the plant virus migrates a long distance from one leaf to an above leaf, and therefore the vascular tissue is also a migration site that leads to systemic infection of a plant. Accordingly, if a gene involved in proliferation or migration of a fungus or plant virus can be introduced into a plant and expressed specifically in the vascular tissue, the plant can be protected from the fungus or plant virus.

A trichome is a floccose outgrowth found on the surface of a leaf, stem, sepal and the like of a plant body. A trichome is involved in secretion and excretion from the surface of a plant body. For example, it is reported that when a plant is exposed to heavy metal (cadmium) stress, the number of trichomes on the surface of leaves increases and crystals containing cadmium or calcium adhere to the surface of the leaves, in other words, that cadmium is excreted by a trichome (Planta, 213 (1), 45-50, 2001, May). A trichome is also the site of first contact for a filamentous fungus, bacterium, insect or the like invading a plant. Further, as a defense against diseases and insect damages, for example, a fluid having antimicrobial activity and a feeding deterrent effect is secreted from a glandular hair or glandular trichome of rugosa rose of the family Rosaceae, one type of trichome. Therefore, if a gene involved in the excretion of a heavy metal or the like, or a gene involved in the secretion of a fluid having antimicrobial activ-

ity or a feeding deterrent effect can be introduced into a plant and expressed specifically in a trichome, a heavy metal can be efficiently excreted from the plant or the plant can be effectively protected against a filamentous fungus, bacterium or insect invading the plant.

As described above, it is desirable that specific gene expression be performed in a vascular tissue or trichome. As a method for performing specific gene expression, a method involving the use of a promoter exhibiting specific promoter activity in a vascular tissue or trichome can be considered. However, a promoter exhibiting promoter activity specifically in both a vascular tissue and a trichome has not been identified at present.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide, for example, a paraquat resistance gene and a vascular tissue- and trichome-specific promoter by identifying and analyzing genes of *Arabidopsis thaliana*.

The present invention accomplishes the aforementioned object by providing the following.

The invention provides a gene encoding a protein of the following (a) or (b):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO: 2;

(b) a protein consisting of an amino acid sequence having a substitution, deletion or addition of one or a plurality of amino acids relative to the amino acid sequence represented by SEQ ID NO: 2 and capable of imparting paraquat resistance.

The invention also provides a protein capable of imparting paraquat resistance encoded by the gene as recited above; a recombinant vector comprising the gene as recited above; a recombinant vector comprising the gene as recited above, wherein the recombinant vector further comprises a foreign gene or a foreign DNA fragment; a transformant having any one of the recombinant vectors as recited above; and a plant body having any one of the recombinant vectors as recited above and having paraquat resistance.

The invention further provides a method for screening for a transgenic plant, comprising introducing a recombinant vector into a plant; wherein the recombinant vector comprises a gene encoding a protein of the following (a) or (b):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO: 2;

(b) a protein consisting of an amino acid sequence having a substitution, deletion or addition of one or a plurality of amino acids relative to the amino acid sequence represented by SEQ ID NO: 2 and capable of imparting paraquat resistance; and wherein the recombinant vector further comprises a foreign gene or a foreign DNA fragment; and screening for a transgenic plant on the basis of paraquat resistance as an indicator.

The invention also provides a method for screening for a transgenic plant, comprising introducing a recombinant vector into a plant; wherein the recombinant vector comprises a gene encoding a protein of the following (a) or (b):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO: 2;

(b) a protein consisting of an amino acid sequence having a substitution, deletion or addition of one or a plurality of amino acids relative to the amino acid sequence represented by SEQ ID NO: 2 and capable of imparting paraquat resistance; and screening for a transgenic plant on the basis of paraquat resistance as an indicator.

In addition, the invention provides a vascular tissue- and trichome-specific promoter comprising DNA of the following (a), (b) or (c):

(a) DNA consisting of the nucleotide sequence represented by SEQ ID NO: 3;

(b) DNA consisting of a nucleotide sequence having a substitution, deletion or addition of one or a plurality of nucleotides relative to the nucleotide sequence represented by SEQ ID NO: 3 and capable of functioning as a vascular tissue- and trichome-specific promoter;

(c) DNA hybridizing under stringent conditions to DNA consisting of the nucleotide sequence represented by SEQ ID NO: 3 and capable of functioning as a vascular tissue- and trichome-specific promoter.

The invention also provides a recombinant vector comprising the vascular tissue- and trichome-specific promoter as recited above; a recombinant vector comprising the vascular tissue- and trichome-specific promoter as recited above, wherein the recombinant vector comprises a foreign gene or a foreign DNA fragment downstream of the vascular tissue- and trichome-specific promoter; a recombinant vector comprising the vascular tissue- and trichome-specific promoter as recited above, wherein the recombinant vector comprises a foreign gene or a foreign DNA fragment downstream of the vascular tissue- and trichome-specific promoter and wherein the foreign gene is a gene encoding a protein of the following (a) or (b):

(a) a protein comprising the amino acid sequence represented by SEQ ID NO: 2;

(b) a protein consisting of an amino acid sequence having a substitution, deletion or addition of one or a plurality of amino acids relative to the amino acid sequence represented by SEQ ID NO: 2 and capable of imparting paraquat resistance; and a transgenic plant having any one of the recombinant vectors as recited above.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with drawings will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 is a photograph of electrophoresis of cDNA derived from an AtMVR gene transformant;

FIG. 2A is a schematic diagram showing the location of an AtMVR gene transformant and a non-transformant in FIGS. 2B and 2C. FIG. 2B is a photograph showing the growth of an AtMVR gene transformant and a non-transformant in a 1/2 MS culture medium without paraquat. FIG. 2C is a photograph showing the growth of an AtMVR gene transformant and a non-transformant in a 1/2 MS culture medium with paraquat;

FIG. 3 is a photomicrograph of an entire transformant containing the GUS gene and an AtMVR promoter histochemically colored with GUS;

FIG. 4 is a photomicrograph of a leaf of a transformant containing the GUS gene and an AtMVR promoter histochemically colored with GUS; and

FIG. 5 is a photomicrograph of a root of a transformant containing the GUS gene and an AtMVR promoter histochemically colored with GUS.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention will be described in detail below.

The gene according to the present invention is a gene encoding the protein of the following (a) or (b):

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- (a) a protein consisting of the amino acid sequence represented by SEQ ID NO: 2; and
 (b) a protein consisting of an amino acid sequence having a substitution, deletion or addition of one or a plurality of amino acids relative to the amino acid sequence represented by SEQ ID NO: 2 and imparting paraquat resistance.

The gene encoding the protein described in the above (a) is a gene (hereafter, referred to as "AtMVR gene") encoding a protein imparting paraquat resistance which consists of the amino acid sequence represented by SEQ ID NO: 2.

The present inventors performed a search on databases having the entire nucleotide sequence of *Arabidopsis thaliana* (for example, GenBank, EMBL, DDBJ, tair: The Arabidopsis Information Resource) based on the nucleotide sequence of the AtMVR gene and found that there are 13 genes homologous to the AtMVR gene (AtMVR 3-1 to AtMVR 3-13) present on the *Arabidopsis thaliana* genome. The nucleotide sequence of each of these AtMVR homologous genes and the putative amino acid sequence encoded by the relevant AtMVR homologous gene are represented by the SEQ ID NOs. listed in Table 1 below. Table 1 also lists the results of homology analysis between the AtMVR gene and each AtMVR homologous gene. The homology analysis was conducted using BLAST P at the amino acid level. Amino acids may be classified based on the chemical properties of their side chains. In the BLOSUM62 amino acid substitution matrix (Proc. Natl. Acad. Sci., 89, 10915-10919, 1992), amino acids are classified into: an amino acid with a mercapto group (C); hydrophilic amino acids that have low molecular weight (S, T, P, A, G); acidic amino acids (N, D, E, Q); basic amino acids (H, R, K); hydrophobic amino acids that have low molecular weights (M, I, L, V); and aromatic amino acids (F, Y, W). In Table 1, the term "Identities" refers to 100% correspondence in terms of amino acids and the term "Positives" refers to the numerical value when amino acids having a positive score in the BLOSUM 62 amino acid substitution matrix are added to those having 100% correspondence (see Bioinformatics (in Japanese), Eds. Okazaki Y. & Bono H. (published by Medical Science International)).

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protein encoded by the AtMVR gene has identity of 89% at the amino acid level to the protein encoded by DSA 5. It is reported that DSA 5 is a gene that expresses upon aging of the petal of lily (*Hemerocallis* hybrid cultivar) (Plant Molecular Biology 40, 237-248, 1999). However, since the protein encoded by DSA 5 has no homology with any known protein, it is unclear which functions the protein has. Accordingly, the AtMVR gene is a novel gene imparting paraquat resistance.

As used herein, the term "paraquat resistance" refers to having resistance to paraquat. More specifically, the term "paraquat-resistant plant" refers to a plant requiring a larger quantity of paraquat than a non-resistant plant in order to obtain a given effect from paraquat. Paraquat is a non-selective and potent herbicide that kills all plants by continuously generating active oxygens in the photochemical system. It is possible to confirm whether the AtMVR gene is a paraquat resistance gene imparting paraquat resistance by examining whether a transformant into which the gene was introduced can grow in the presence of paraquat.

The gene encoding the protein described in the above (b) is a gene encoding a protein consisting of an amino acid sequence having a substitution, deletion or addition of one or a plurality of amino acids (for example, 1 to 10, or 1 to 5) relative to the amino acid sequence represented by SEQ ID NO: 2 and imparting paraquat resistance.

Once the nucleotide sequence of the gene according to the present invention has been determined, it is then possible to obtain the gene according to the present invention by chemical synthesis, or by polymerase chain reaction (hereafter, referred to as "PCR") employing as a template a clone that has been cloned, or by performing hybridization employing a DNA fragment having the nucleotide sequence as a probe. Further, it is possible to synthesize a mutant of the gene according to the present invention having equivalent functions as those prior to mutation by a technique such as site-directed mutagenesis.

Examples of the method for introducing a mutation into the gene according to the present invention include a known method such as the Kunkel method or the gapped duplex method or a method in accordance with such methods. For

TABLE 1

Name of AtMVR homologous gene	Nucleotide sequence	Amino acid sequence	Identities (%)	Positives (%)
AtMVR3-1	SEQ ID NO: 4	SEQ ID NO: 5	57	70
AtMVR3-2	SEQ ID NO: 6	SEQ ID NO: 7	55	69
AtMVR3-3	SEQ ID NO: 8	SEQ ID NO: 9	39	56
AtMVR3-4	SEQ ID NO: 10	SEQ ID NO: 11	39	55
AtMVR3-5	SEQ ID NO: 12	SEQ ID NO: 13	37	54
AtMVR3-6	SEQ ID NO: 14	SEQ ID NO: 15	36	52
AtMVR3-7	SEQ ID NO: 16	SEQ ID NO: 17	34	52
AtMVR3-8	SEQ ID NO: 18	SEQ ID NO: 19	34	51
AtMVR3-9	SEQ ID NO: 20	SEQ ID NO: 21	37	58
AtMVR3-10	SEQ ID NO: 22	SEQ ID NO: 23	25	44
AtMVR3-11	SEQ ID NO: 24	SEQ ID NO: 25	33*	51*
AtMVR3-12	SEQ ID NO: 26	SEQ ID NO: 27	25	41
AtMVR3-13	SEQ ID NO: 28	SEQ ID NO: 29	22	41

*The comparison with AtMVR3-11 shows the homology result for comparison with a partial sequence of AtMVR3-11.

As shown in Table 1, the homology of AtMVR with the 13 AtMVR homologous genes ranged from 22 to 57% for Identities and from 41 to 70% for Positives. These AtMVR homologous genes are considered to impart paraquat resistance in the same manner as the AtMVR gene.

Further, the AtMVR gene has homology to a senescence-associated protein, DSA 5 (GenBank accession number AF082030) (Plant Molecular Biology 40, 237-248, 1999). The result of homology analysis using BLAST X showed the

example, introduction of a mutation can be performed using a kit for introducing a mutation (for example, Mutant-K (manufactured by TAKARA, Inc.), or Mutant-G (manufactured by TAKARA, Inc.)) utilizing site-directed mutagenesis or using LA PCR in vitro Mutagenesis series kit manufactured by TAKARA, Inc.

A protein imparting paraquat resistance according to the present invention is the protein encoded by the gene according to the present invention. For example, the gene according

to the present invention is integrated into a vector derived from *Escherichia coli* or the like, and *E. coli* is then transformed with the obtained recombinant vector. Thereafter, the protein according to the present invention can be obtained by extracting the protein synthesized within *E. coli*.

Further, a recombinant vector according to the present invention is a recombinant vector comprising the gene according to the present invention. The recombinant vector according to the present invention can be obtained by inserting the gene according to the present invention into an appropriate vector. A vector used for inserting the gene according to the present invention is not particularly limited as long as it is capable of replication within a host, and examples thereof include a plasmid, a shuttle vector, and a helper plasmid. In addition, when the vector itself is not capable of replication, a DNA fragment that is capable of replication by a method such as insertion into the chromosome of a host may be used.

Examples of plasmid DNA include a plasmid derived from *E. coli* (pBI221 and the like, for example, pET system such as pET30b, pBR system such as pBR322 and pBR325, pUC system such as pUC118, pUC119, pUC18 and pUC19, pBluescript, and pBI221), a plasmid derived from *Bacillus subtilis* (for example, pUB110 and pTP5), a binary plasmid derived from *Agrobacterium tumefaciens* (for example, pBI system derived from pBIN19, pBI101, or pBI121), a plasmid derived from yeast (for example, YEp system such as YEp13, or YCp system such as YCp50) or the like. Examples of phage DNA include λ phage (Charon 4A, Charon 21A, EMBL3, EMBL4, λ gt10, λ gt11, λ ZAP and the like). Further, an animal virus vector such as retrovirus or vaccinia virus, a plant virus vector such as cauliflower mosaic virus, or an insect virus vector such as baculovirus can also be used.

To insert the gene according to the present invention into a vector, a method may be used in which cDNA of the gene according to the present invention is first cleaved using an appropriate restriction enzyme and then inserted into a restriction enzyme site or multicloning site of an appropriate vector DNA and ligated into the vector. Further, a method may be used in which a homologous region is respectively provided in one part of a vector and cDNA of the gene according to the present invention, and the vector and the cDNA are connected by an in vitro method using PCR or the like or an in vivo method using yeast or the like.

A recombinant vector according to the present invention can also include a foreign gene or a foreign DNA fragment in addition to the gene according to the present invention. A method for inserting a foreign gene or a foreign DNA fragment into a vector is the same as the method for inserting a DNA fragment according to the present invention into a vector. Any gene or DNA fragment may be used as a foreign gene or a foreign DNA fragment. Thus, the gene according to the present invention can be used as a selective marker gene to indicate paraquat resistance, for example, as with an antibiotic resistance gene for kanamycin or hygromycin or the like.

A transformant according to the present invention is a transformant having the recombinant vector according to the present invention. The transformant according to the present invention can be obtained by introducing the recombinant vector according to the present invention into a host. A host is not particularly limited as long as it is capable of expressing the gene according to the present invention, however a plant is preferred. When the host is a plant, it is possible to obtain a transgenic plant in the manner described below.

A "plant" to be transformed in the present invention may be any of: a whole plant, a plant organ (for example, leaf, petal, stem, root, or seed), plant tissue (for example, epidermis, phloem, parenchyma, or xylem) or a plant culture cell.

Examples of the plant that can be used in the transformation include, but are not limited to, a plant belonging to the family Poaceae, Brassicaceae, Solanaceae, or Leguminosae (see below).

Poaceae: *Oryza sativa*, *Zea mays*
 Brassicaceae: *Arabidopsis thaliana*
 Solanaceae: *Nicotiana tabacum*
 Leguminosae: *Glycine max*

The recombinant vector according to the present invention can be introduced into a plant by a conventional transformation method such as, for example, the electroporation method, *Agrobacterium* method, particle gun method, or PEG method.

For example, when using the electroporation method, the recombinant vector according to the present invention is introduced into a host by conducting the treatment using an electroporation apparatus equipped with a pulse controller under conditions of a voltage of 500 to 1600 V, at 25 to 1000 μ F, for 20 to 30 msec.

When using the particle gun method, the whole plant, a plant organ or plant tissue itself may be used without any treatment, a section thereof may be prepared and then used, or protoplast may be prepared and used. The prepared sample can then be treated using a gene transfer device (for example, PDS-1000/He manufactured by Bio-Rad Inc.). Although the treatment conditions may vary depending on the plant or sample used, the treatment is normally conducted at a pressure of approximately 450 to 2000 psi and a distance of approximately 3 to 12 cm.

A method using the Ti plasmid or Ri plasmid of *Agrobacterium* takes advantage of a characteristic whereby, when a bacterium belonging to the genus *Agrobacterium* infects a plant, one part of plasmid DNA possessed by the bacterium is transferred into the genome of the plant. This method can thus be used to introduce the gene according to the present invention into a plant host. Among the bacteria belonging to the genus *Agrobacterium*, when *Agrobacterium tumefaciens* infects a plant, it causes the formation of a tumor that is referred to as "crown gall." Further, when *Agrobacterium rhizogenes* infects a plant, it incites generation of a capillary root. These are caused by a region referred to as a "T-DNA (Transferred DNA) region" of a Ti plasmid or Ri plasmid transferring into a plant at the time of infection to be integrated into the genome of the plant. Accordingly, the DNA to be integrated into a plant genome is first inserted into the T-DNA region of a Ti plasmid or Ri plasmid, and then the DNA can be integrated into the plant genome by infecting the plant host with a bacterium of the genus *Agrobacterium*.

Examples of the method for transforming a bacterium of the genus *Agrobacterium* into a plant host include the above described electroporation method, patent gun method and PEG method, as well as an in planta method. Examples of the in planta method include a direct *Agrobacterium* inoculation method and an infiltration method.

Tumor tissue or shoot, capillary root or the like obtained as the result of the transformation can be used without any treatment for cell culture, tissue culture or organ culture. Alternatively, it can be regenerated in a plant body by administration of a plant hormone (auxin, cytokinin, gibberellin, abscisic acid, ethylene, brassinolide or the like) of an appropriate concentration using a conventional plant tissue culture method.

The gene according to the present invention may also be introduced into a plant by utilizing a plant virus as a vector. Examples of the plant virus that can be used include cauliflower mosaic virus. First, the viral genome is inserted into a vector derived from *E. coli* or the like to produce a recombi-

nant, and then the gene according to the present invention is inserted into the viral genome. The viral genome modified in this manner is subsequently cleaved from the recombinant using a restriction enzyme, and the gene according to the present invention can then be introduced into a plant host by inoculating the viral genome into the plant host.

In addition to introduction into a plant host as described above, the recombinant vector according to the present invention may also be introduced into bacteria belonging to the genus *Escherichia* such as *E. coli*, the genus *Bacillus* such as *Bacillus subtilis*, or the genus *Pseudomonas* such as *Pseudomonas putida*, as well as yeast such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, animal cells such as COS cell or CHO cell, and insect cells such as Sf9. When using a bacterium such as *E. coli* or yeast or the like as a host, it is preferable that the recombinant vector according to the present invention is capable of autonomous replication in the bacterium and that it is comprised of a promoter, a ribosome binding sequence, a transcription termination sequence and the gene according to the present invention. It may also comprise a gene regulating the promoter.

The method for introducing the recombinant vector according to the present invention into a bacterium is not particularly limited as long as it is a method that can introduce DNA into a bacterium, and for example a method using calcium ion or the electroporation method may be mentioned.

The method for introducing the recombinant vector according to the present invention into yeast is not particularly limited as long as it is a method that can introduce DNA into yeast, and for example the electroporation method, spheroplast method and lithium acetate method may be mentioned.

When using an animal cell as a host, monkey cell COS-7, Vero, Chinese hamster ovary cell (CHO cell), mouse L-cells or the like can be used. The method for introducing the recombinant vector according to the present invention into an animal cell is not particularly limited as long as it is a method that can introduce DNA into an animal cell, and for example the electroporation method, calcium phosphate method and lipofection method may be mentioned.

When using an insect cell as a host, an Sf9 cell or the like can be used. The method for introducing the recombinant vector according to the present invention into an insect cell is not particularly limited as long as it can introduce DNA into an insect cell, and for example the calcium phosphate method, lipofection method and electroporation method may be mentioned.

It is possible to confirm whether or not the gene according to the present invention has been integrated into a host by using the PCR method, Southern hybridization method, Northern hybridization method or the like. For example, PCR can be conducted after preparing DNA from the transformant and designing a DNA-specific primer. Next, the amplification product is subjected to agarose gel electrophoresis, polyacrylamide gel electrophoresis or capillary electrophoresis or the like, and the product thereof is then stained with ethidium bromide, SYBR Green solution or the like. Thereafter, whether or not transformation has occurred can be confirmed by the detection of the amplification product as a single band. It is also possible to detect the amplification product after conducting PCR using a primer that has been labeled previously with a fluorescent dye or the like. In addition, a method may be employed in which the amplification product is bound to a solid phase of a microplate or the like to enable confirmation of the amplification product by fluorescence or enzyme reaction or the like.

A plant body according to the present invention is one having a recombinant vector comprising the gene according to the present invention and having paraquat resistance. As used herein, the term "plant body" refers to a whole plant transformed with a recombinant vector comprising the gene according to the present invention. The plant body according to the present invention can be obtained by introducing the above recombinant vector into a plant cell or the like and regenerating a transgenic plant body from the obtained transgenic plant cell. As a regeneration method, a method may be employed in which transformed cells in a callus form are transferred to a culture medium in which the type and concentration of hormones have been modified and allowed to culture, and an adventitious embryo is allowed to form to obtain a complete plant body. Examples of the culture medium to be used include LS medium and MS medium. Introduction of a recombinant vector into a plant cell or the like can be performed by a method similar to the method described above.

In the plant body according to the present invention, a protein imparting paraquat resistance that is encoded by the gene according to the present invention is overexpressed throughout the whole plant body. Thus, the plant body according to the present invention can have resistance to paraquat.

A method of screening for transgenic plants according to the present invention is a method in which the recombinant vector according to the present invention is introduced into plants and paraquat resistance is used as an indicator to screen for transgenic plants. Transformation can be verified by employing the gene according to the present invention as a selective marker gene to indicate paraquat resistance. Examples of the screening method include a method in which plants transformed by the recombinant vector according to the present invention are grown in a paraquat-containing medium and the screening is carried out based on variations in the life and death as well as growth of the plants. The concentration of paraquat used for the screening may vary depending on the species and size of plants and the like, however, for example, when *Arabidopsis thaliana* is used as a host, paraquat may be present in a medium at a concentration of preferably 0.1 to 3.0 μM , more preferably 1.0 to 3.0 μM , and most preferably 3.0 μM . A non-transgenic plant, i.e., a wild-type plant, develops chlorosis and dies in a paraquat-containing medium. In contrast, a plant transformed with the recombinant vector according to the present invention remains green even in a paraquat-containing medium. Thus, it is possible to verify a clear difference in growth in a paraquat-containing medium between a non-transgenic plant and a plant transformed with the recombinant vector according to the present invention.

When employing antibiotic resistance or herbicide resistance as an indicator, false positivity may be observed at the screening stage because of the existence of a difference in sensitivity among the plant. In contrast, paraquat is a non-selective and potent herbicide that can kill all plants. Consequently, in the method of screening transgenic plants according to the present invention, false positivity is not observed in the screening stage. Further, according to the method of screening transgenic plants according to the present invention, resistance can be effectively confirmed at an early stage of growth.

The promoter according to the present invention is a vascular tissue- and trichome-specific promoter comprising the DNA of the following (a), (b) or (c):

(a) DNA consisting of the nucleotide sequence represented by SEQ ID NO: 3;

(b) DNA consisting of a nucleotide sequence having a substitution, deletion or addition of one or a plurality of nucleotides relative to the nucleotide sequence represented by SEQ ID NO: 3 and functioning as a vascular tissue- and trichome-specific promoter; and

(c) DNA hybridizing under stringent conditions to DNA consisting of the nucleotide sequence represented by SEQ ID NO: 3 and functioning as a vascular tissue- and trichome-specific promoter.

The vascular tissue- and trichome-specific promoter described in the above (a) is a vascular tissue- and trichome-specific promoter found in an untranslated region on the 5'-upstream side of the AtMVR gene and consists of the nucleotide sequence represented by SEQ ID NO: 3. The promoter described in (a) can be determined by performing a search based on approximately 3,000 nucleotides on the 5'-upstream side of the AtMVR gene on a database having the complete nucleotide sequence for *Arabidopsis thaliana*.

As used herein, the term "vascular tissue- and trichome-specific promoter" refers to a promoter exhibiting activity specific to a vascular tissue and trichome of a plant. The term "vascular tissue" refers to a fascicular tissue system that differentiates through each organ of pteridophytes and spermatophytes, such as the stem, leaf, and root. Xylem and phloem are the components of the vascular tissue, and they function as pipes to transport water and internal substances throughout the plant. Meanwhile, the term "trichome" refers to a floccose outgrowth existing on the surface of a leaf, stem, or sepal of a plant body. A trichome participates in secretion and excretion from the plant body surface.

The activity of a vascular tissue- and trichome-specific promoter can be determined in accordance with a conventional method. For example, an expression vector having a reporter gene operably linked thereto downstream of a promoter may be constructed. Next, an appropriate plant is transformed with the expression vector. The obtained transformant is then cultured under predetermined conditions, and the expression amount of the reporter gene in a vascular tissue and trichome may be determined at the mRNA or protein level to enable the measurement of promoter activity under the relevant conditions. Further, when the reporter gene is the β -Glucuronidase (GUS) gene, the specificity of promoter activity in a vascular tissue and trichome can be determined by observing the histochemical coloring caused by the expressed GUS.

For example, as a method of the histochemical coloring using GUS, a method may be mentioned in which a reaction mixture containing 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (X-Gluc) as a GUS substrate is added to a tissue of a transformant in which the GUS gene has been introduced. When the GUS gene is expressed, X-Gluc is de-esterified to generate an indoxyl derivative monomer, and this monomer is oxidation-polymerized with air to form a blue indigotin pigment. In a transformed cell or tissue, this blue pigment accumulates to exhibit a blue color.

Further, a specified untranslated region on the 5'-upstream side of the AtMVR gene can be readily obtained by conducting PCR employing genome extracted from *Arabidopsis thaliana* as a template and using primers that are complementary to the nucleotide sequences on both ends of the region.

The promoter according to the present invention may be the nucleotide sequence represented by SEQ ID NO: 3, more specifically, the entire untranslated region on the 5'-upstream side, or it may be one part of DNA consisting of the nucleotide sequence represented by SEQ ID NO: 3 in so far as it exhibits a function as a vascular tissue- and trichome-specific promoter.

The vascular tissue- and trichome-specific promoter described in the above (b) consists of a nucleotide sequence having a substitution, deletion or addition of one or a plurality of nucleotides (for example, 1 to 10, or 1 to 5) relative to the nucleotide sequence represented by SEQ ID NO: 3 and functions as a vascular tissue- and trichome-specific promoter.

The vascular tissue- and trichome-specific promoter described in the above (c) hybridizes under stringent conditions to DNA consisting of the nucleotide sequence represented by SEQ ID NO: 3 and functions as a vascular tissue- and trichome-specific promoter.

As used herein, the term "stringent conditions" refers to, for example, when using probe DNA labeled with phosphorus-32, hybridization in a hybridization solution consisting of 5 \times SSC (0.75 M NaCl, 0.75 M sodium citrate), 5 \times Denhardt's reagent (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin) and 0.1% sodium dodecyl sulphate (SDS) at a temperature between 45 and 68 $^{\circ}$ C., preferably 60 to 68 $^{\circ}$ C. Further, in the washing step, washing is performed in a washing solution consisting of 2 \times SSC and 0.1% SDS at a temperature between 45 and 55 $^{\circ}$ C., and more preferably in a washing solution consisting of 0.1 \times SSC and 0.1% SDS at a temperature between 45 and 55 $^{\circ}$ C. When using probe DNA enzymatically labelled using the AlkPhos direct labeling module kit (Amersham Biotech), hybridization may be conducted in a hybridization solution (containing 0.5 M NaCl and 4% blocking reagent) having the composition described in the manual accompanying the kit at a temperature between 55 to 75 $^{\circ}$ C. Further, in a washing step, washing may be conducted in a primary washing solution (containing 2 M urea) in accordance with the instructions in the manual accompanying the kit at a temperature between 55 to 75 $^{\circ}$ C., and in a secondary washing solution at room temperature. Other detection techniques may also be used, in which case the conditions may be the standard conditions for the relevant detection technique.

Once the nucleotide sequence of the promoter according to the present invention has been determined, it is then possible to obtain the promoter according to the present invention by chemical synthesis, or by PCR employing a cloned probe as a template, or by performing hybridization employing a DNA fragment having the nucleotide sequence as a probe. Further, it is possible to synthesize a mutant of the promoter according to the present invention having equivalent functions as those prior to mutation by a technique such as site-directed mutagenesis.

Examples of the method for introducing a mutation into the promoter according to the present invention include a known method such as the Kunkel method or the gapped duplex method or a method in accordance with such methods. For example, the introduction of a mutation can be performed using a kit for introducing a mutation (for example, Mutant-K or Mutant-G (both manufactured by TAKARA, Inc.) utilizing site-directed mutagenesis or using LA PCR in vitro Mutagenesis series kit manufactured by TAKARA, Inc.

A recombinant vector according to the present invention comprising the promoter according to the present invention can be obtained by inserting the promoter according to the present invention into an appropriate vector. A vector for inserting the promoter according to the present invention is not particularly limited as long as it is capable of replication within a host, and examples thereof include a plasmid, a shuttle vector, and a helper plasmid. In addition, when the vector itself is not capable of replication, a DNA fragment that is capable of replication by a method such as insertion into the chromosome of a host may be used.

Examples of plasmid DNA include a plasmid derived from *E. coli* (pBI221 and the like, for example, pET system such as pET30b, pBR system such as pBR322 and pBR325, pUC system such as pUC118, pUC119, pUC18 and pUC19, pBluescript, and pBI221), a plasmid derived from *Bacillus subtilis* (for example, pUB110 and pTP5), a binary plasmid derived from *Agrobacterium tumefaciens* (for example, pBI system derived from pBIN19, pBI101, or pBI121), a plasmid derived from yeast (for example, YEp system such as YEp13, or YCp system such as YCp50) or the like. Examples of phage DNA include λ phage (Charon 4A, Charon 21A, EMBL3, EMBL4, λ gt10, λ gt11, λ ZAP and the like). Further, an animal virus vector such as retrovirus or vaccinia virus, or an insect virus vector such as baculovirus can also be used.

To insert the promoter according to the present invention into a vector, a method may be used in which purified DNA is first cleaved with an appropriate restriction enzyme and then inserted into a restriction enzyme site or multicloning site of an appropriate vector DNA and ligated to the vector. Further, a method may also be used in which a homologous region is respectively provided in one part of a vector and the promoter according to the present invention, and the vector and promoter are ligated by an in vitro method using PCR or the like or an in vivo method using yeast or the like.

The recombinant vector according to the present invention comprising the promoter according to the present invention can further include a foreign gene or a foreign DNA fragment that is inserted downstream of the promoter according to the present invention. A method for inserting a foreign gene or a foreign DNA fragment is the same as a method for inserting the promoter according to the present invention into a vector.

In the recombinant vector according to the present invention comprising the promoter according to the present invention, examples of a foreign gene to be inserted downstream of the promoter according to the present invention include any foreign gene, and specific examples include a gene involved in transport of water or internal substances or in cell proliferation, a gene involved in proliferation or transport of bacteria or a plant virus, a gene involved in discharge of a heavy metal or the like, or a gene involved in secretion of a liquid having antimicrobial activity or a feeding deterrent effect. More specifically, the gene may be a gene for transporter or pump, a gene encoding a PR-protein (Pathogenesis related protein) (chitinase, peroxidase or the like), a defensin family gene, a phytoalexin synthesis gene or a repellent pheromone synthesis gene of a pest insect or the like. As further examples of a foreign gene, the gene according to the present invention described above, the AtMVR gene, may be mentioned.

Examples of the foreign DNA fragment to be inserted downstream of the promoter according to the present invention include antisense RNA or a ribozyme in which the RNA itself is functioning.

The transgenic plant according to the present invention is a transgenic plant having the recombinant vector according to the present invention comprising the promoter according to the present invention. The transgenic plant according to the present invention can be obtained by introducing the recombinant vector according to the present invention comprising the promoter according to the present invention into a plant. A transgenic plant can be obtained in the manner described below.

A "plant" to be transformed in the present invention may be any of: a whole plant, a plant organ having a vascular tissue and/or trichome (for example, leaf, petal, stem, root, or seed), plant tissue (for example, epidermis, phloem, parenchyma, or xylem) or a plant culture cell. Examples of the plant that can be used in the transformation include, but are not limited to, a

plant belonging to the family Poaceae, Brassicaceae, Solanaceae, or Leguminosae (see below).

Poaceae: *Oryza sativa*, *Zea mays*

Brassicaceae: *Arabidopsis thaliana*

Solanaceae: *Nicotiana tabacum*

Leguminosae: *Glycine max*

The recombinant vector according to the present invention comprising the promoter according to the present invention can be introduced into a plant by a conventional transformation method such as, for example, the electroporation method, *Agrobacterium* method, particle gun method, or PEG method.

For example, when using the electroporation method the recombinant vector according to the present invention comprising the promoter according to the present invention is introduced into a host by the treatment using an electroporation apparatus equipped with a pulse controller under conditions of a voltage of 500 to 1600 V, at 25 to 1000 μ F, for 20 to 30 msec.

When using the particle gun method, the whole plant, a plant organ or plant tissue itself may be used without any treatment, a section thereof may be prepared and then used, or protoplast may be prepared and used. The prepared sample can then be treated using a gene transfer device (for example, PDS-1000/He manufactured by Bio-Rad Inc.). Although the treatment conditions may vary depending on the plant or sample used, the treatment is normally conducted at a pressure of approximately 450 to 2000 psi and a distance of approximately 3 to 12 cm.

The method using the Ti plasmid or Ri plasmid of *Agrobacterium* takes advantage of a characteristic whereby, when a bacterium belonging to the genus *Agrobacterium* infects a plant, one part of plasmid DNA possessed by the bacterium is transferred into the genome of the plant. This method can thus be used to introduce the promoter according to the present invention and a foreign gene or foreign DNA fragment into a plant host. Among the bacteria belonging to the genus *Agrobacterium*, when *Agrobacterium tumefaciens* infects a plant, it causes the formation of a tumor that is referred to as "crown gall." Further, when *Agrobacterium rhizogenes* infects a plant, it incites the generation of a capillary root. These are caused by a region referred to as a "T-DNA (Transferred DNA) region" of a Ti plasmid or Ri plasmid transferring into a plant at the time of infection to be integrated into the genome of the plant. Accordingly, the DNA to be integrated into a plant genome is first inserted into the T-DNA region of a Ti plasmid or Ri plasmid, and then the DNA can be integrated into the plant genome by infecting the plant host with a bacterium of the genus *Agrobacterium*.

Examples of the method for transforming a bacterium of the genus *Agrobacterium* into a plant host include the above described electroporation method, particle gun method and PEG method, as well as an in planta method. Examples of the in planta method include a direct *Agrobacterium* inoculation method and an infiltration method.

The tumor tissue or shoot, capillary root or the like obtained as the result of transformation can be used without any treatment for cell culture, tissue culture or organ culture. Alternatively, it can be regenerated in a plant body by administration of a plant hormone (auxin, cytokinin, gibberellin, abscisic acid, ethylene, brassinolide or the like) of an appropriate concentration using a plant tissue culture method known in the prior art.

Further, the promoter according to the present invention and a foreign gene or foreign DNA fragment can be introduced into a plant by utilizing a plant virus as a vector. Examples of the plant virus that can be used herein include cauliflower mosaic virus. First, the viral genome is inserted into a vector derived from *E. coli* or the like to produce a recombinant, and then the promoter according to the present

invention and the foreign gene or foreign DNA fragment is inserted into the viral genome. The viral genome modified in this manner is subsequently cleaved from the recombinant using a restriction enzyme, and the promoter according to the present invention and the foreign gene or foreign DNA fragment can be introduced into a plant host by inoculating the viral genome into the plant host.

The transgenic plant according to the present invention produced in the above manner can specifically express the foreign gene or foreign DNA fragment in a vascular tissue and trichome using the promoter according to the present invention.

The vascular tissue is a location involved in transporting water and internal substances as well as cell proliferation in a plant. Thus, when a gene involved in transporting water or internal substances or in cell proliferation is introduced as a foreign gene into the transgenic plant according to the present invention, the transport of water and internal substances or cell proliferation in the plant can be regulated. The vascular tissue is also a site where wilt disease fungus infecting plants of the family Solanaceae proliferates and transfers. When a plant virus infects a plant, the plant virus migrates a long distance from one leaf to an above leaf and therefore the vascular tissue is also a migration site that leads to systemic infection of a plant. Thus, when a gene involved in proliferation or migration of a fungus or plant virus is introduced as a foreign gene into the transgenic plant according to the present invention, the plant can be protected from the fungus or plant virus.

Meanwhile, a trichome is involved in secretion and excretion from the surface of a plant body. For example, it is reported that when a plant is exposed to heavy metal (cadmium) stress, the number of trichomes on the surface of leaves increases and crystals containing cadmium or calcium adhere to the surface of the leaves, in other words, that cadmium is excreted by a trichome (Planta, 213 (1), 45-50, 2001, May). A trichome is also the site of first contact for a filamentous fungus, bacterium, insect or the like invading a plant. Further, as a defense against diseases and insect damages, for example, a fluid having antimicrobial activity and a feeding deterrent effect is secreted from a glandular hair or glandular trichome of rugosa rose of the family Rosaceae, one type of trichome. Therefore, when a gene involved in the excretion of a heavy metal or the like, or a gene involved in secretion of a fluid having antimicrobial activity or a feeding deterrent effect is introduced as a foreign gene into the transgenic plant according to the present invention, heavy metal can be efficiently excreted from the plant or the plant can be effectively protected against a filamentous fungus, bacterium or insect invading the plant.

Further, when the gene according to the present invention is introduced as a foreign gene into the transgenic plant according to the present invention, resistance to paraquat can be imparted by promoting the transportation and excretion and the like of paraquat incorporated in the plant body.

EXAMPLES

The present invention will be explained in detail further below with reference to the following examples. However, the examples are not intended to limit the technical scope of the invention.

Example 1

Isolation of Paraquat Resistance Gene

In this example, Weigel T-DNA lines acquired from Nottingham *Arabidopsis* Stock Center (School of Biosciences, University of Nottingham, Sutton Bonington Campus,

Loughborough, LE12 5RD, United Kingdom) were used as activation tag lines of *Arabidopsis thaliana*.

(1) Screening of Individuals Capable of Growing in Paraquat-containing Medium Using Activation-tagged Lines of *Arabidopsis thaliana* (Weigel T-DNA Lines)

Seeds of Weigel T-DNA lines were sterilely inoculated in 1/2 MS agar (1%) culture medium (2.3 g/l of Murashige and Skoog Plant Salt Mixture (manufactured by Wako Pure Chemical Industries Ltd.), 1.5 mg/l of thiamine hydrochloride, 2.5 mg/l of nicotinic acid, 0.25 mg/l of pyridoxine hydrochloride, 1.5% of sucrose, 1% of agar) containing 3 μM of paraquat (methyl viologen, manufactured by Sigma Chemical Co.), and cultured at 22° C. under irradiation of light of 60 μE/m²/s (cycle of 16 hrs photoperiod/8 hrs dark period). Approximately 10 days after culture, individuals growing in the paraquat-containing medium were screened.

(2) Estimation of Insertion Sites of T-DNA from the Screened Activation-tagged Lines by the TAIL-PCR Method

Seeds of Weigel T-DNA lines from which screened individuals originated were planted in a pot containing vermiculite (manufactured by Asahi Kagaku Kogyo Co., Ltd.) and grown for approximately one month at 23° C. under a light intensity of 100 μE/m²/s with a photoperiod condition of 16 hrs photoperiod/8 hrs dark period.

Genome DNA was prepared from leaves of cultivated individuals using the DNeasy Plant Mini Kit (manufactured by QIAGEN), and three types of specific primers (TL1: SEQ ID NO: 31; TL2: SEQ ID NO: 32; TL3: SEQ ID NO: 33) were designed for the vicinity of a T-DNA left sequence (T-DNA left border: SEQ ID NO: 30) of an activation-tagging vector (pSKI015: GenBank accession No. AF187951) used with the Weigel T-DNA lines. TAIL-PCR (Shokubutsu No PCR Jikken Purotokoru (Protocols of PCR Experiments for Plants), (Eds. Shimamoto K. & Sasaki T.), New Edition, 2000, pp 83-89, Shujunsha Co., Ltd., Tokyo; Genomics, 25, 674-681, 1995; Plant J., 8, 457-463, 1995) was then performed using the specific primers and a random primer 1 (SEQ ID NO: 34) and the PCR reaction mixture and reaction conditions described below to amplify genome DNA bordering the T-DNA. In SEQ ID NO: 34, n represents a, g, c, or t (location: 1 and 11), s represents g or c (location: 7), and w represents a or t (location: 8 and 13).

The composition of the reaction mixture and the PCR conditions for the first-round PCR are listed in tables 2 and 3.

TABLE 2

Template (genome DNA):	10 ng
10x PCR buffer (manufactured by TAKARA BIO Inc.):	2 μl
2.5 mM dNTPs (manufactured by TAKARA BIO Inc.):	1.6 μl
First specific primer (TL1: SEQ ID NO: 31):	3 pmol
Random primer 1 (SEQ ID NO: 34):	80 pmol
AmpliTaq (manufactured by Applied Biosystems):	0.8 units
Total volume	20 μl

TABLE 3

#1:	94° C. (1 min)/95° C. (1 min)
#2:	94° C. (1 min)/65° C. (1 min)/72° C. (3 min) × 5 cycles
#3:	94° C. (1 min)/25° C. (3 min) → to 72° C. at 3 min/72° C. (3 min) × 1 cycle
#4:	94° C. (30 sec)/68° C. (1 min)/72° C. (3 min) 94° C. (30 sec)/68° C. (1 min)/72° C. (3 min) 94° C. (30 sec)/44° C. (1 min)/72° C. (3 min) × 14 cycles
#5:	72° C. (5 min)

The composition of the reaction mixture and the PCR conditions for the second-round PCR are listed in tables 4 and 5.

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TABLE 4

Template (a 50-fold dilution of product of first-round PCR):	1 μ l
10x PCR buffer:	2 μ l
250 μ M dNTPs:	2 μ l
Second specific primer (TL2; SEQ ID NO: 32):	4 pmol
Random primer 1 (SEQ ID NO: 34):	60 pmol
AmpliTaq:	0.6 units
Total volume	20 μ l

TABLE 5

#6:	94° C. (30 sec)/64° C. (1 min)/72° C. (3 min)
	94° C. (30 sec)/64° C. (1 min)/72° C. (3 min)
	94° C. (30 sec)/44° C. (1 min)/72° C. (3 min) \times 10 cycles
#5	72° C. (5 min)

The composition of the reaction mixture and the PCR conditions for the third-round PCR are listed in tables 6 and 7.

TABLE 6

Template (a 50-fold dilution of product of second-round PCR):	1 μ l
10x PCR buffer:	10 μ l
2.5 mM dNTPs:	1 μ l
Third specific primer (TL3; SEQ ID NO: 33):	30 pmol
Random primer 1 (SEQ ID NO: 34):	300 pmol
AmpliTaq:	3 units
Total volume	100 μ l

TABLE 7

#7:	94° C. (1 min)/44° C. (1 min)/72° C. (3 min) \times 20 cycles
#5	72° C. (5 min)

Next, after subjecting the reaction products from the second-round PCR and third-round PCR to electrophoresis on agarose gel, the presence or absence of amplification and the specificity of the reaction products were verified.

Further, using the specific primer TL3 (SEQ ID NO: 33), the amplification product of the third-round PCR was directly sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and the nucleotide sequence was then determined using the ABI PRISM 310 genetic analyzer (Applied Biosystems). As a result, 278-bp sequence information was obtained (SEQ ID NO: 35). In SEQ ID NO: 35, n represents a, g, c, or t (location: 13, 35, 73, 108, 156, 190, 198 and 201). A search was performed for the obtained sequence on databases having the entire nucleotide sequence of *Arabidopsis thaliana*, and it was found that the insertion site is located at 77240 bp of BAC clone F17123.

(3) Isolation of cDNA of Paraquat Resistance Gene

Seeds of *Arabidopsis thaliana* (*Arabidopsis thaliana* ecotype Columbia (Col-0)) were planted in a pot containing vermiculite (Asahi Kagaku Kogyo Co., Ltd.) and allowed to grow for approximately one month at 23° C. under a light intensity of 100 μ E/m²/s with a photoperiod condition of 16 hrs photoperiod/8 hrs dark period.

After growing, leaves of individuals were frozen using liquid nitrogen. Subsequently, total RNA was extracted using the RNeasy Plant Mini Kit (manufactured by QIAGEN). Thereafter, cDNA was synthesized from the extracted total RNA using the ProSTAR First Strand RT-PCR Kit (manufactured by STRATAGEN).

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Based on the sequence of a putative open reading frame (ORF) gene present within an adjacent 10 kb of a structural gene having the nucleotide sequence (SEQ ID NO: 35) obtained in the above (2), a primer 141dF (SEQ ID NO: 36) and a primer 141dR (SEQ ID NO: 37) were designed for the putative structural gene, and PCR was then performed using these primers and the following reaction mixture (Table 8) containing Takara EX-Taq (manufactured by TAKARA BIO Inc.) employing the above synthesized cDNA as a template.

TABLE 8

Template (cDNA):	50 ng
10x Ex Taq buffer (TAKARA BIO Inc.):	2 μ l
dNTPs:	200 μ M
Each primer:	0.2 μ M
Takara EX-Taq:	1 unit
Total volume	20 μ l

Thirty cycles of 94° C. (30 sec)/55° C. (30 sec)/72° C. (60 sec) were employed as the reaction conditions.

The amplification product was cloned into the pGEM-T Easy vector (manufactured by Promega), and the nucleotide sequence was then determined using the ABI PRISM 310 genetic analyzer (Applied Biosystems). As a result, a cDNA fragment of 857 bp was obtained (SEQ ID NO: 1). This cDNA fragment was designated as AtMVR gene, and the pGEM-T Easy vector containing AtMVR gene was designated as pAt-MVR. The amino acid sequence encoded by the AtMVR gene is shown in SEQ ID NO: 2.

Example 2

Search for AtMVR Homologous Gene with Respect to AtMVR Gene

The search was made on databases having the entire nucleotide sequence of *Arabidopsis thaliana* based on the nucleotide sequence of the AtMVR gene and found that in addition to the nucleotide sequence of the AtMVR gene there are 13 AtMVR homologous genes on the *Arabidopsis thaliana* genome.

The respective AtMVR homologous genes were designated as AtMVR3-1 to AtMVR3-13. The nucleotide sequence of each of the AtMVR homologous genes and the putative amino acid sequence encoded by the relevant AtMVR homologous gene are shown by the SEQ ID NOs. listed in Table 9 below. Table 9 also lists the results of homology analysis between the AtMVR gene and each AtMVR homologous gene. The homology analysis was conducted using BLAST P at the amino acid level. The term "Identities" refers to 100% correspondence in terms of amino acids. Amino acids may be classified based on the chemical properties of their side chains. In the BLOSUM62 amino acid substitution matrix, amino acids are classified into: an amino acid with a mercapto group (C); hydrophilic amino acids that have low molecular weights (S, T, P, A, G); acidic amino acids (N, D, E, Q); basic amino acids (H, R, K); hydrophobic amino acids that have low molecular weights (M, I, L, V); and aromatic amino acids (F, Y, W). In Table 9, the term "Identities" refers to 100% correspondence in terms of amino acids and the term "Positives" refers to the numerical value when amino acids having a positive score in the BLOSUM 62 amino acid substitution matrix are added to those having 100% correspondence.

TABLE 9

Name of AtMVR homologous gene	Nucleotide sequence	Amino acid sequence	Identities (%)	Positives (%)
AtMVR3-1	SEQ ID NO: 4	SEQ ID NO: 5	57	70
AtMVR3-2	SEQ ID NO: 6	SEQ ID NO: 7	55	69
AtMVR3-3	SEQ ID NO: 8	SEQ ID NO: 9	39	56
AtMVR3-4	SEQ ID NO: 10	SEQ ID NO: 11	39	55
AtMVR3-5	SEQ ID NO: 12	SEQ ID NO: 13	37	54
AtMVR3-6	SEQ ID NO: 14	SEQ ID NO: 15	36	52
AtMVR3-7	SEQ ID NO: 16	SEQ ID NO: 17	34	52
AtMVR3-8	SEQ ID NO: 18	SEQ ID NO: 19	34	51
AtMVR3-9	SEQ ID NO: 20	SEQ ID NO: 21	37	58
AtMVR3-10	SEQ ID NO: 22	SEQ ID NO: 23	25	44
AtMVR3-11	SEQ ID NO: 24	SEQ ID NO: 25	33*	51*
AtMVR3-12	SEQ ID NO: 26	SEQ ID NO: 27	25	41
AtMVR3-13	SEQ ID NO: 28	SEQ ID NO: 29	22	41

*The comparison with AtMVR3-11 shows the result of homology comparison with a partial sequence of AtMVR3-11.

As shown in Table 9, homology between AtMVR and the 13 AtMVR homologous genes ranged from 22 to 57% for Identities and from 41 to 70% for Positives.

Example 3

Construction of AtMVR Expression Vector for Plant and Production of AtMVR Transgenic Plant

The transformation techniques applied herein were in accordance with a vector system described by Pellegrineschi et al. (Biochemical Society Transitions 23, 247-250, 1995) based on the *Agrobacterium* gene transport system outlined by Hinchee et al. (Plant Cell and Tissue Culture, pp. 231-270, Eds. I. K. Vasil, T. A Thorpe, Kluwer Academic Publisher, 1994).

(1) Construction of AtMVR Expression Vector for Plant

The AtMVR gene sequence was excised from pAtMVR using SacI/SacII and subcloned into pBlueScript (STRATAGENE). Subsequently, a fragment containing the AtMVR gene sequence was cleaved with XbaI/SacI and introduced at XbaI/SacI site that is present downstream of the CaMV 35S promoter of pBI121 (manufactured by Clontech). The resulting vector was used below as an AtMVR expression vector for plant.

(2) Production of AtMVR Transgenic Plant

The AtMVR expression vector for plant produced in the above (1) was introduced into *Agrobacterium tumefaciens* LBA4404 strain by the electroporation method (Plant Molecular Biology Manual, Second Edition, B. G. Stanton, A. S. Robbert, Kluwer Academic Publishers, 1994). Subsequently, the *Agrobacterium tumefaciens* having the AtMVR expression vector for plant introduced therein was introduced into wild-type *Arabidopsis thaliana* ecotype Col-0 by an infiltration method described by Clough et al. (The Plant Journal 16: 735-743, 1998).

Transformants were screened in a kanamycin-containing medium, and a T3 generation plant (homozygous line having 1 AtMVR gene introduced) was produced by self-pollination.

Next, the amount of the introduced AtMVR gene expressed above and a non-transformant were respectively planted in pots containing vermiculite (Asahi Kagaku Kogyo Co., Ltd.) and allowed to grow for approximately one month under a light intensity of 100 $\mu\text{E}/\text{m}^2/\text{s}$ at 23° C. with a photoperiod condition of 16 hrs photoperiod/8 hrs dark period.

After growing, total RNA was extracted from the transformant and the wild-type *Arabidopsis thaliana* ecotype Col-0 non-transformant using the RNeasy Plant Mini Kit (QIAGEN). Thereafter, 1 μg of RNA was subjected to reverse transcription using the ProSTAR First Strand RT-PCR Kit (STRATAGEN). PCR was then performed using the following reaction mixture (Table 10) containing Takara EX-Taq (TAKARA BIO) employing 1/50 volume of the synthesized cDNA as a template and using primers for the AtMVR gene (primer 141d1 (SEQ ID NO: 38) and primer 141d2 (SEQ ID NO: 39)).

TABLE 10

PCR reaction mixture:	
Template (cDNA):	50 ng
10x Ex Taq buffer (TAKARA BIO Inc.):	2 μl
dNTPs:	200 μM
Each primer:	0.2 μM
Takara EX-Taq:	1 unit
Total volume	20 μl

Thirty cycles of 94° C. (30 sec)/55° C. (30 sec)/72° C. (60 sec) were employed as the reaction conditions.

The amplification products were subjected to electrophoresis on agarose gel. FIG. 1 shows the results of electrophoresis. As can be seen from FIG. 1, in comparison to the non-transformant, the produced transformant overexpressed the AtMVR gene.

Example 4

Evaluation of Paraquat Resistance of AtMVR Gene Transformant

Seeds derived from the produced AtMVR gene transformant and non-transformant (wild-type *Arabidopsis thaliana* ecotype Col-0) were sterilely inoculated in 1/2 MS agar (1%) medium (2.3 μl of Murashige and Skoog Plant Salt Mixture (Wako Pure Chemical Industries Ltd.), 1.5 mg/l of thiamine hydrochloride, 2.5 mg/l of nicotinic acid, 0.25 mg/l of pyridoxine hydrochloride, 1.5% of sucrose) containing 3 μM of paraquat (methyl viologen (Sigma Chemical Co.)), and cultured for eight days at 22° C. under irradiation of light of 60 $\mu\text{mol}/\text{m}^2/\text{s}$ (cycle of 16 hrs photoperiod/8 hrs dark period). After culture, the growth of germinated individuals was evaluated. The results are shown in FIG. 2, wherein FIG. 2B

is a photograph showing growth of an AtMVR gene transformant and a non-transformant in a 1/2 MS culture medium without paraquat, and FIG. 2C is a photograph showing growth of an AtMVR gene transformant and a non-transformant in a 1/2 MS culture medium with paraquat. FIG. 2A is a schematic diagram showing the location of the AtMVR gene transformant and the non-transformant in FIGS. 2B and 2C.

As can be seen from FIG. 2B, the results showed that in the culture medium without paraquat, the AtMVR gene transformant exhibited the same growth as the non-transformant. Meanwhile, as can be seen from FIG. 2C, in a medium containing paraquat the non-transformant developed chlorosis and died, i.e. growth was remarkably inhibited, while in contrast the seedling of the AtMVR gene transformant was able to grow. Thus, it was confirmed that in a medium without paraquat, the AtMVR gene transformant exhibited the same growth as a non-transformant regardless of expression of the AtMVR gene, and also that in a medium with paraquat, the AtMVR gene transformant had clearly greater paraquat resistance than the non-transformant.

Example 5

Isolation of Vascular Tissue/Trichome-specific Promoter

Seeds of *Arabidopsis thaliana* (*Arabidopsis thaliana* ecotype Columbia (Col-0)) were planted in pots containing vermiculite (Asahi Kagaku Kogyo Co., Ltd.) and allowed to grow for approximately one month under a light intensity of 100 $\mu\text{E}/\text{m}^2/\text{s}$ at 23° C., under a photoperiod condition of 16 hrs photoperiod/8 hrs dark period.

After growing, genome DNA was prepared from leaves of individuals using DNeasy Plant Mini Kit (QIAGEN). Next, PCR was performed using the following reaction mixture (Table 11) containing Takara EX-Taq (TAKARA BIO Inc.) employing the obtained genome DNA as a template and using a primer 141dpF (SEQ ID NO: 40) and a primer 141dpR (SEQ ID NO: 41) based on the AtMVR gene fragment (SEQ ID NO: 35) described in above Example 1 under the reaction conditions described below.

TABLE 11

Template genome DNA:	50 ng
10x Ex Taq buffer (TAKARA BIO Inc.):	2 μl
dNTPs:	200 μM
Each primer:	0.2 μM
Takara EX-Taq:	1 unit
Total volume	20 μl

The reaction conditions were thirty cycles of 94° C. (30 sec)/55° C. (30 sec)/72° C. (60 sec).

The amplification product was cloned into pGEM-T Easy vector (Promega Corp.) and the nucleotide sequence was then determined using the ABI PRISM 310 genetic analyzer (Applied Biosystems). As a result, an AtMVR promoter of 1722 bp (SEQ ID NO: 3) was obtained.

Example 6

Analysis of Tissue Specificity of Vascular Tissue/Trichome-specific Promoter

(1) Construction of Expression Vector Having AtMVR Promoter

The AtMVR promoter was excised from the pGEM-T Easy vector having the AtMVR promoter (SEQ ID NO: 3) pro-

duced in Example 5 using HindIII/PstI and then subcloned into the upstream region of CaMV 35S promoter of pBI221 (Clontech). The resulting vector was treated with PstI/SmaI to remove the CaMV 35S promoter, and the ends were blunted using DNA T4 polymerase (TAKARA BIO Inc.) and allowed to self-ligate. As a result, the AtMVR promoter was ligated into the vector upstream of the β -Glucuronidase (GUS) gene. Subsequently, a fragment containing the AtMVR promoter and the GUS gene was excised from the above vector using HindIII/EcoRI, and then substituted for CaMV 35S promoter- β -GUS gene of pBI121 (Clontech). The vector thus obtained was employed as an expression vector having the AtMVR promoter for use below.

(2) Production of Transgenic Plant

In a similar manner to Example 3(2), the above expression vector having the AtMVR promoter was introduced into *Agrobacterium tumefaciens* LBA4404 strain, and this was then introduced into wild-type *Arabidopsis thaliana* ecotype Col-0 by the infiltration method. Thereafter, transformants were screened in a kanamycin-containing medium, and a T3 generation plant was produced by self-pollination.

(3) Analysis of Tissue Specificity of AtMVR Promoter

Seeds derived from the transformant produced in the above (2) were sterilely inoculated in 1/2 MS agar (1%) medium (2.3 g/l of Murashige and Skoog Plant Salt Mixture (Wako Pure Chemical Industries Ltd.), 1.5 mg/l of thiamine hydrochloride, 2.5 mg/l of nicotinic acid, 0.25 mg/l of pyridoxine hydrochloride, 1.5% of sucrose), and cultured at 22° C. under irradiation of light of 60 $\mu\text{E}/\text{m}^2/\text{s}$ (cycle of 16 hrs photoperiod/8 hrs dark period) for approximately 7 days.

After culture, transformants that grew were fixed with acetone. A reaction mixture containing 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (X-Gluc) was added to the fixed tissue so as to immerse the entire tissue. The composition of the reaction mixture was: 1.9 mM of X-Gluc, 0.5 mM of $\text{K}_3\text{Fe}(\text{CN})_6$, 0.5 mM of $\text{K}_4\text{Fe}(\text{CN})_6$, and 0.3% of Triton X-100.

The container was then sealed and incubated overnight in a 37° C. incubator. Thereafter, 70% ethanol was added to the mixture to terminate the reaction, and coloring was observed. (Shokubutsu No Saibo Wo Miru Jikken Purotokoru (Protocols of Experiments for Observing Cells of Plants), Eds. Fukuda H., Nishimura M., & Nakamura K., (1997), pp 71-79, Shujunsha Co., Ltd., Tokyo). The results are shown in FIGS. 3 to 5, wherein FIGS. 3 to 5 are photomicrographs of an entire transformant, a leaf of a transformant, and a root of a transformant, respectively.

As can be seen from FIGS. 3 to 5, specific coloring was observed in the vascular tissue and trichome. Thus, it was confirmed that the obtained AtMVR promoter (SEQ ID NO: 3) is a transcriptional promoter having tissue-specific transcriptional activity in a vascular tissue and trichome.

Free Text for Sequence Listing

SEQ ID NOS: 31 to 41 are primers.

In SEQ ID NO: 34, n represents a, g, c, or t (location: 1 and 11), s represents g or c (location: 7), and w represents a or t (location: 8 and 13).

In SEQ ID NO: 35, n represents a, g, c, or t (location: 13, 35, 73, 108, 156, 190, 198 and 201).

INDUSTRIAL APPLICABILITY

According to the present invention there is provided a paraquat resistance gene and a vascular tissue- and trichome-specific promoter. A paraquat resistance gene according to the

present invention is capable of imparting resistance that is specific to paraquat without affecting growth regulation that undergoes control by the generation of active oxygens under various environments.

Further, the vascular tissue- and trichome-specific promoter according to the present invention enables the regulation of gene expression in a vascular tissue and trichome of a plant.

 SEQUENCE LISTING

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cgacgcaatg cgagagattc ctcgacaaac ccatgatcgc tctcggtggtt ttcctcatga      180
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<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 2

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  20          25          30
Lys Ala Thr Thr Gln Cys Glu Arg Phe Leu Asp Lys Pro Met Ile Ala
  35          40          45
Leu Gly Val Phe Leu Met Ile Ile Ala Ile Ala Gly Val Val Gly Ser
  50          55          60
Cys Cys Arg Val Thr Trp Leu Leu Trp Ser Tyr Leu Phe Val Met Phe
  65          70          75          80
Phe Leu Ile Leu Ile Val Leu Cys Phe Thr Ile Phe Ala Phe Val Val
  85          90          95
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  115         120         125
  
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Cys Tyr Asn Leu Glu Leu Val Thr Ala Asn His Thr Val Ser Asp Phe
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Tyr Lys Glu Asp Leu Thr Ala Phe Glu Ser Gly Cys Cys Lys Pro Ser
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Asn Asp Cys Asp Phe Thr Tyr Ile Thr Ser Thr Thr Trp Asn Lys Thr
 180 185 190

Ser Gly Thr His Lys Asn Ser Asp Cys Gln Leu Trp Asp Asn Glu Lys
 195 200 205

His Lys Leu Cys Tyr Asn Cys Lys Ala Cys Lys Ala Gly Phe Leu Asp
 210 215 220

Asn Leu Lys Ala Ala Trp Lys Arg Val Ala Ile Val Asn Ile Ile Phe
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Cys Cys Arg Val Thr Trp Leu Leu Trp Val Tyr Leu Phe Val Met Phe
 65          70          75          80
Leu Leu Ile Leu Leu Val Phe Cys Ile Thr Val Phe Ala Phe Val Val
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Thr Asn Lys Gly Ala Gly Glu Ala Ile Glu Gly Lys Gly Tyr Lys Glu
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Leu	Leu	Ile	Leu	Leu	Gly	Phe	Cys	Phe	Thr	Ile	Phe	Ala	Phe	Ala	Val
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Thr	Asn	Arg	Gly	Ala	Gly	Glu	Val	Ile	Ser	Asp	Arg	Gly	Tyr	Lys	Glu
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Tyr	Lys	Ser	Asn	Leu	Asn	Ala	Leu	Gln	Ser	Gly	Cys	Cys	Lys	Pro	Ser
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Pro	Gly	Pro	Tyr	Lys	Asn	Glu	Asp	Cys	Asn	Val	Trp	Asp	Asn	Lys	Pro
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Gly	Thr	Leu	Cys	Tyr	Asp	Cys	Glu	Ala	Cys	Lys	Ala	Gly	Leu	Leu	Asp
	210					215					220				
Asn	Ile	Lys	Asn	Ser	Trp	Lys	Lys	Val	Ala	Lys	Val	Asn	Ile	Val	Phe
	225				230					235					240
Leu	Ile	Phe	Leu	Ile	Ile	Val	Tyr	Ser	Val	Gly	Cys	Cys	Ala	Phe	Arg
			245					250						255	
Asn	Asn	Arg	Lys	Arg	Ser	Trp									
			260												

<210> SEQ ID NO 8

<211> LENGTH: 984

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 8

atgagatcga gaagtaacct tataggtctc ataaacttct tcactttcct cctgtcgatt	60
cctatcctcg gcggtggaat atggcttagc agccgagcta actcaaccga ttgcctcaga	120
ttctccagt gccactcat tatcatcgga atatcaatca tggtcatatc tttagccgga	180
atcgccggag cttgttacca aaacaagttc ctcatgtggc tttacctttt caccatgttc	240
tttgaatcg ctgctcttat aggattcaca atcttcgctt acgtagttag tgataaaggc	300
tcaggccggt ttgtgatgaa ccgtcgggat cttgattatt atctcaatga ttattccggt	360
tggtaaagg accgtgtcac agataatgga tattggagag atatcggatc gtgtgttaga	420
gattctggag tttgtaagaa gattggaaga gatttaaag gtgttccaga aactgtcat	480
atgttttact tcagaaatct ttctcctgtt gagtccggat gttgcaagcc gccaacagat	540
tgtggctata cgtacgtgaa cgagacagtg tggattccgg gaggagaaat ggtgggaccg	600
aaccggact gtatgtgtg gaacaatgac cagagactac tctgttacca atgcagctct	660
tgtaaagccg gtgttcttgg tagcttgaag aagagttgga gaaaagtctc ggtgatcaac	720
atcgtggttg tgatcactat tgttatcttc tatgtcatcg cgtgtgcggc ttaccagaat	780

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gttaagagga tgtataatga cgaaccggtc ggtgaggcta ggatgaccaa tctcacccta 840
gtcattttca aatttaagga gatttttgta cagtttttct tcggaattgt gtttttatta 900
ctctttaatg gtttaatggt ctggttggtg aatgataaat ttgcttttag tgttttcttc 960
tttgatgatg ttacatatgc atga 984

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<210> SEQ ID NO 9
<211> LENGTH: 327
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

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<400> SEQUENCE: 9

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```

Met Arg Ser Arg Ser Asn Leu Ile Gly Leu Ile Asn Phe Phe Thr Phe
 1           5           10          15
Leu Leu Ser Ile Pro Ile Leu Gly Gly Ile Trp Leu Ser Ser Arg
 20          25          30
Ala Asn Ser Thr Asp Cys Leu Arg Phe Leu Gln Trp Pro Leu Ile Ile
 35          40          45
Ile Gly Ile Ser Ile Met Val Ile Ser Leu Ala Gly Ile Ala Gly Ala
 50          55          60
Cys Tyr Gln Asn Lys Phe Leu Met Trp Leu Tyr Leu Phe Thr Met Phe
 65          70          75          80
Phe Val Ile Ala Ala Leu Ile Gly Phe Thr Ile Phe Ala Tyr Val Val
 85          90          95
Thr Asp Lys Gly Ser Gly Arg Phe Val Met Asn Arg Arg Tyr Leu Asp
100         105         110
Tyr Tyr Leu Asn Asp Tyr Ser Gly Trp Leu Lys Asp Arg Val Thr Asp
115         120         125
Asn Gly Tyr Trp Arg Asp Ile Gly Ser Cys Val Arg Asp Ser Gly Val
130         135         140
Cys Lys Lys Ile Gly Arg Asp Leu Asn Gly Val Pro Glu Thr Ala His
145         150         155         160
Met Phe Tyr Phe Arg Asn Leu Ser Pro Val Glu Ser Gly Cys Cys Lys
165         170         175
Pro Pro Thr Asp Cys Gly Tyr Thr Tyr Val Asn Glu Thr Val Trp Ile
180         185         190
Pro Gly Gly Glu Met Val Gly Pro Asn Pro Asp Cys Met Leu Trp Asn
195         200         205
Asn Asp Gln Arg Leu Leu Cys Tyr Gln Cys Ser Ser Cys Lys Ala Gly
210         215         220
Val Leu Gly Ser Leu Lys Lys Ser Trp Arg Lys Val Ser Val Ile Asn
225         230         235         240
Ile Val Val Val Ile Ile Leu Val Ile Phe Tyr Val Ile Ala Cys Ala
245         250         255
Ala Tyr Gln Asn Val Lys Arg Met Tyr Asn Asp Glu Pro Val Gly Glu
260         265         270
Ala Arg Met Thr Asn Leu Ile Leu Val Ile Phe Lys Phe Lys Glu Ile
275         280         285
Leu Val Gln Phe Phe Phe Gly Ile Val Phe Leu Leu Leu Phe Asn Gly
290         295         300
Leu Met Val Cys Cys Cys Asn Asp Lys Phe Ala Phe Ser Val Phe Phe
305         310         315         320
Phe Gly Tyr Val Thr Tyr Ala
325

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-continued

<210> SEQ ID NO 10
 <211> LENGTH: 858
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 10

```

atgagaacaa gcaaccatct cataggttta gtcaacttcc tcactttcct cctctcaata    60
ccaatcctcg gcggtggaat atggttaagc agccgagcta actccaccga ctgtttaaga    120
ttccttcaat ggctctcat cgtcatcgga atctcaatca tggtcgtatc ttagctgga    180
ttcgtggag cttgttaccg taacaagttc cttatgtggc tatacctagt agtcatgctt    240
ctcatcatcg ctgctcttat cggtttcacg atcttcgctt acgcggttac agataaagga    300
tccggtcgaa ccgtacttaa ccgggggttat cttgactatt atcttgaaga ttactctggt    360
tggttgaaag atcgagtttc tgatgatagc tattggggta aaattagttc ttgtcttaga    420
gattctggtg cttgtagaaa gattggaaga aattttaatg gtgtacctga aactgctgat    480
atgttcttcc ttagaagact tagccctgtt gagtccggtt gttgcaagcc accaacagat    540
tgcggttttt catatgtgaa tgagaccgga tgggacacga gaggagggat gataggaccg    600
aaccaggact gtatggtgtg gagcaacgac cagagcatgc tctgttatca gtgtagtctt    660
tgtaaagctg gtgttcttgg gagtttgaag aagagttgga gaaaagtatc ggtgatcaac    720
attgtggtac ttatcattct agttatcttt tacgttatcg cttatgcagc ttataggaat    780
gtcaagagga tcgataacga tgaaccggct ggtgaagcta ggatgacaaa atcacatcct    840
agtcatttcc atctttga                                858
  
```

<210> SEQ ID NO 11
 <211> LENGTH: 285
 <212> TYPE: PRT
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 11

```

Met Arg Thr Ser Asn His Leu Ile Gly Leu Val Asn Phe Leu Thr Phe
  1           5           10          15
Leu Leu Ser Ile Pro Ile Leu Gly Gly Gly Ile Trp Leu Ser Ser Arg
  20          25          30
Ala Asn Ser Thr Asp Cys Leu Arg Phe Leu Gln Trp Pro Leu Ile Val
  35          40          45
Ile Gly Ile Ser Ile Met Val Val Ser Leu Ala Gly Phe Ala Gly Ala
  50          55          60
Cys Tyr Arg Asn Lys Phe Leu Met Trp Leu Tyr Leu Val Val Met Leu
  65          70          75          80
Leu Ile Ile Ala Ala Leu Ile Gly Phe Ile Ile Phe Ala Tyr Ala Val
  85          90          95
Thr Asp Lys Gly Ser Gly Arg Thr Val Leu Asn Arg Gly Tyr Leu Asp
  100         105         110
Tyr Tyr Leu Glu Asp Tyr Ser Gly Trp Leu Lys Asp Arg Val Ser Asp
  115         120         125
Asp Ser Tyr Trp Gly Lys Ile Ser Ser Cys Leu Arg Asp Ser Gly Ala
  130         135         140
Cys Arg Lys Ile Gly Arg Asn Phe Asn Gly Val Pro Glu Thr Ala Asp
  145         150         155         160
Met Phe Phe Leu Arg Arg Leu Ser Pro Val Glu Ser Gly Cys Cys Lys
  165         170         175
  
```

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Pro Pro Thr Asp Cys Gly Phe Ser Tyr Val Asn Glu Thr Gly Trp Asp
 180 185 190

Thr Arg Gly Gly Met Ile Gly Pro Asn Gln Asp Cys Met Val Trp Ser
 195 200 205

Asn Asp Gln Ser Met Leu Cys Tyr Gln Cys Ser Ser Cys Lys Ala Gly
 210 215 220

Val Leu Gly Ser Leu Lys Lys Ser Trp Arg Lys Val Ser Val Ile Asn
 225 230 235 240

Ile Val Val Leu Ile Ile Leu Val Ile Phe Tyr Val Ile Ala Tyr Ala
 245 250 255

Ala Tyr Arg Asn Val Lys Arg Ile Asp Asn Asp Glu Pro Ala Gly Glu
 260 265 270

Ala Arg Met Thr Lys Ser His Pro Ser His Phe His Leu
 275 280 285

<210> SEQ ID NO 12
 <211> LENGTH: 849
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 12

```

atgtacagat tcagcaacac agttattggg gtcttaaacc ttctcacctt actagcctcg      60
attccaatca tcggaaccgc tttatacaag gcaagaagca gcacgacatg tgaaaacttc      120
ctccagacgc cgctacttgt tataggattc atcatactca tagtttcctt tgcgggatc      180
ataggagcct gcttcaacgt ggcattgggt ctttgggtgt acttagtggt catgatcttc      240
ctcatcgcta ccctaattgg tctaacgcta tttggtctgg tggtgacgag ccaaggaggt      300
ggagtggaag tgccagggag gatttataaa gagtataggc ttggagacta tcatccatgg      360
ttgagagaga gagttaggga tcctgagtat tggaaactcca ttagaagctg tatcttgagt      420
tccaagactt gtactaagat tgagtccttg actacacttg attattcca aagagacatg      480
acttctgttc agtcgggatg ttgtaagcca cgcacggcgt gtacgtacga agctggagta      540
gtggacggag gaggagattg cttcagatgg aacaatggag tggagatggt atgctacgag      600
tgcgatgctt gcaaggtcgg tgttctcgaa gagatccgtc tcgactggag aaagttatcg      660
gttgtcaaca ttctcgtcct cgtcctctc atcgcggtct acgccgctgg ttgctgcgcc      720
ttccacaaca ctgccaccgc agctcatcct taccatccat ctgatgataa ccgcatgacc      780
agagtccgtc ctcgttggga ctattactgg tggagatggt ggcacgaaaa gaaagagcag      840
ctttactaa
    
```

<210> SEQ ID NO 13
 <211> LENGTH: 282
 <212> TYPE: PRT
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 13

Met Tyr Arg Phe Ser Asn Thr Val Ile Gly Val Leu Asn Leu Leu Thr
 1 5 10 15

Leu Leu Ala Ser Ile Pro Ile Ile Gly Thr Ala Leu Tyr Lys Ala Arg
 20 25 30

Ser Ser Thr Thr Cys Glu Asn Phe Leu Gln Thr Pro Leu Leu Val Ile
 35 40 45

Gly Phe Ile Ile Leu Ile Val Ser Leu Ala Gly Phe Ile Gly Ala Cys
 50 55 60

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Phe Asn Val Ala Trp Ala Leu Trp Val Tyr Leu Val Val Met Ile Phe
 65 70 75 80
 Leu Ile Ala Thr Leu Met Gly Leu Thr Leu Phe Gly Leu Val Val Thr
 85 90 95
 Ser Gln Gly Gly Gly Val Glu Val Pro Gly Arg Ile Tyr Lys Glu Tyr
 100 105 110
 Arg Leu Gly Asp Tyr His Pro Trp Leu Arg Glu Arg Val Arg Asp Pro
 115 120 125
 Glu Tyr Trp Asn Ser Ile Arg Ser Cys Ile Leu Ser Ser Lys Thr Cys
 130 135 140
 Thr Lys Ile Glu Ser Trp Thr Thr Leu Asp Tyr Phe Gln Arg Asp Met
 145 150 155 160
 Thr Ser Val Gln Ser Gly Cys Cys Lys Pro Pro Thr Ala Cys Thr Tyr
 165 170 175
 Glu Ala Gly Val Val Asp Gly Gly Gly Asp Cys Phe Arg Trp Asn Asn
 180 185 190
 Gly Val Glu Met Leu Cys Tyr Glu Cys Asp Ala Cys Lys Ala Gly Val
 195 200 205
 Leu Glu Glu Ile Arg Leu Asp Trp Arg Lys Leu Ser Val Val Asn Ile
 210 215 220
 Leu Val Leu Val Leu Leu Ile Ala Val Tyr Ala Ala Gly Cys Cys Ala
 225 230 235 240
 Phe His Asn Thr Arg His Ala Ala His Pro Tyr His Pro Ser Asp Asp
 245 250 255
 Asn Arg Met Thr Arg Val Arg Pro Arg Trp Asp Tyr Tyr Trp Trp Arg
 260 265 270
 Trp Trp His Glu Lys Lys Glu Gln Leu Tyr
 275 280

<210> SEQ ID NO 14

<211> LENGTH: 810

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 14

```

atgctttaa gcaacaatgt aattggtgc ataaacttca tcaccgtcct cctctccatt 60
ccggtcatcg gcgccgaat ctggctagcc ataggaacag taaactcatg cgtcaagctt 120
cttcaatggc cagtaataat cctcggagtc ttaatcctct tagtgggtct cgctggtttc 180
attggagggg tttggagaat cacatggcct cttgttgttt acttaatcgc catgcttatt 240
ctcattgtac ttttgggttg ccttgtcgga tttatttaca tggttaccat aagaggctct 300
ggtcatccag aaccaagtag agcttatcct gagtatagtc ttcaagattt ctctggttgg 360
ttacgtagaa gagttcagag atcttataaa tgggaaagga ttcgtacttg tttgagtaca 420
actaccattt gccttgaact aatcagaga tacactttgg ctcaagattt cttcaatgct 480
catcttgatc coattcaatc tggttgctgc aagcccccaa caaatgtgg attcacattt 540
gttaatccta cttattggat aagtccata gatatgtctg ctgatatgga ttgtctaaat 600
tggagcaatg accaaaacac tttgtgttac acttgtgatt cttgtaaagc cggcttgctc 660
gcaaattaa aggtagattg gttaaaagcg gatattcttc tactcttggc gcttatcgga 720
ttgattatcg tctacattat cgggtgctgc gcattccgta atgcggaaac tgaggatatt 780
ttcaggaagt acaagcaggg ttataactga 810

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<210> SEQ ID NO 15
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 15

Met Pro Leu Ser Asn Asn Val Ile Gly Cys Ile Asn Phe Ile Thr Val
 1             5             10             15

Leu Leu Ser Ile Pro Val Ile Gly Ala Gly Ile Trp Leu Ala Ile Gly
 20             25             30

Thr Val Asn Ser Cys Val Lys Leu Leu Gln Trp Pro Val Ile Ile Leu
 35             40             45

Gly Val Leu Ile Leu Leu Val Gly Leu Ala Gly Phe Ile Gly Gly Phe
 50             55             60

Trp Arg Ile Thr Trp Leu Leu Val Val Tyr Leu Ile Ala Met Leu Ile
 65             70             75             80

Leu Ile Val Leu Leu Gly Cys Leu Val Gly Phe Ile Tyr Met Val Thr
 85             90             95

Ile Arg Gly Ser Gly His Pro Glu Pro Ser Arg Ala Tyr Leu Glu Tyr
100            105            110

Ser Leu Gln Asp Phe Ser Gly Trp Leu Arg Arg Arg Val Gln Arg Ser
115            120            125

Tyr Lys Trp Glu Arg Ile Arg Thr Cys Leu Ser Thr Thr Thr Ile Cys
130            135            140

Pro Glu Leu Asn Gln Arg Tyr Thr Leu Ala Gln Asp Phe Phe Asn Ala
145            150            155            160

His Leu Asp Pro Ile Gln Ser Gly Cys Cys Lys Pro Pro Thr Lys Cys
165            170            175

Gly Phe Thr Phe Val Asn Pro Thr Tyr Trp Ile Ser Pro Ile Asp Met
180            185            190

Ser Ala Asp Met Asp Cys Leu Asn Trp Ser Asn Asp Gln Asn Thr Leu
195            200            205

Cys Tyr Thr Cys Asp Ser Cys Lys Ala Gly Leu Leu Ala Asn Ile Lys
210            215            220

Val Asp Trp Leu Lys Ala Asp Ile Phe Leu Leu Leu Ala Leu Ile Gly
225            230            235            240

Leu Ile Ile Val Tyr Ile Ile Gly Cys Cys Ala Phe Arg Asn Ala Glu
245            250            255

Thr Glu Asp Ile Phe Arg Lys Tyr Lys Gln Gly Tyr Thr
260            265

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<210> SEQ ID NO 16
<211> LENGTH: 813
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 16

atggcgtag cgaataactt aacggcgata ctcaacttac tagcggtact ctgttcata    60
ccaataacgg cgtcaggtag atggctagct tcaaagccag acaacgagtg tgtcaatctc    120
ctccgttgcc cgttgctcgt cctcggcggt ctcacccctc tcgtctccgc cacaggettc    180
atcggcgccct acaagtacaa ggaaactcta ctggcgggtt acttgctgtg tatggcgata    240
ttgatcggac ttttctgtgt ggttcttata tttgcattcg tcgtgacccg gcccgatgga    300
tcgtatcggg ttccgggtag aggttataaa gaggataggc ttgaagggtt ctgcaattgg    360
cttaaggaga acgttggtga ttccaagaac tggggaaggc taagggttg tttggctgat    420

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actaatgttt gtctaaact caaccaagaa ttcatacccg cggatcagtt cttctcctcc 480
tctaagatca ctctctcca gtccggctgc tgcaaaccac caaccgcatg tggtacaaac 540
tttgtgaacc caacactgtg gctaaatcca accaatatgg ctgcagacgc agactgttac 600
ttatggagca atgaccaaaag ccagctttgt tacaattgca actcatgcaa agctgggtta 660
ttgggaaacc ttagaaaaga atggcgtaaa gcaaatctca tacttatcat cacagtcggt 720
gttctcatat gggtttatgt tattgcttgt agcgcgttta ggaatgetca gactgaggat 780
ctcttcgcga aatacaaaca aggttgggtc taa 813

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<210> SEQ ID NO 17
<211> LENGTH: 270
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

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<400> SEQUENCE: 17

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Met Ala Leu Ala Asn Asn Leu Thr Ala Ile Leu Asn Leu Leu Ala Leu
 1           5           10          15
Leu Cys Ser Ile Pro Ile Thr Ala Ser Gly Ile Trp Leu Ala Ser Lys
 20          25          30
Pro Asp Asn Glu Cys Val Asn Leu Leu Arg Trp Pro Val Val Val Leu
 35          40          45
Gly Val Leu Ile Leu Val Val Ser Ala Thr Gly Phe Ile Gly Ala Tyr
 50          55          60
Lys Tyr Lys Glu Thr Leu Leu Ala Val Tyr Leu Cys Cys Met Ala Ile
 65          70          75          80
Leu Ile Gly Leu Leu Val Val Leu Ile Phe Ala Phe Val Val Thr
 85          90          95
Arg Pro Asp Gly Ser Tyr Arg Val Pro Gly Arg Gly Tyr Lys Glu Tyr
100         105         110
Arg Leu Glu Gly Phe Ser Asn Trp Leu Lys Glu Asn Val Val Asp Ser
115         120         125
Lys Asn Trp Gly Arg Leu Arg Ala Cys Leu Ala Asp Thr Asn Val Cys
130         135         140
Pro Lys Leu Asn Gln Glu Phe Ile Thr Ala Asp Gln Phe Phe Ser Ser
145         150         155         160
Ser Lys Ile Thr Pro Leu Gln Ser Gly Cys Lys Pro Pro Thr Ala
165         170         175
Cys Gly Tyr Asn Phe Val Asn Pro Thr Leu Trp Leu Asn Pro Thr Asn
180         185         190
Met Ala Ala Asp Ala Asp Cys Tyr Leu Trp Ser Asn Asp Gln Ser Gln
195         200         205
Leu Cys Tyr Asn Cys Asn Ser Cys Lys Ala Gly Leu Leu Gly Asn Leu
210         215         220
Arg Lys Glu Trp Arg Lys Ala Asn Leu Ile Leu Ile Ile Thr Val Val
225         230         235         240
Val Leu Ile Trp Val Tyr Val Ile Ala Cys Ser Ala Phe Arg Asn Ala
245         250         255
Gln Thr Glu Asp Leu Phe Arg Lys Tyr Lys Gln Gly Trp Val
260         265         270

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<210> SEQ ID NO 18
<211> LENGTH: 816
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

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<400> SEQUENCE: 18

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atgtttcgag ttagcaattt catggttggc ctagcaaaca cattggtgat gttagtgggc    60
gcttcggcca ttggttattc gatttacatg ttcgttcacc aaggcgtcac tgattgtgaa    120
tctgccatte ggataccact tctcacgacc ggactcatcc tcttcttggc gtctttgtctc    180
ggagtgattg gatcttgttt caaggagaat ttggcaatgg tttcctactt gatcatattg    240
tttgggggca ttggtgcatt gatgatttcc tccatatttc tcttcttggc gaccaacaaa    300
ggagccggtc gtgtggtgtc cggtcgaggg tataaagagt accggacggt ggatttctcg    360
acgtggctta atgggttcgt tgggtggaag agatgggttg ggataaggtc ttgtttggct    420
gaggctaacg tttgtgatga tttgagtgat ggtcgtgtta gtcagatcgc tgatgcgttt    480
tatcacaaga acttgtctcc catccagtca ggttgttcta agccaccatc ggattgcaac    540
ttcgagtta gaaacgcgac gttctggata ccgccgagca aaaacgaaac ggcagttgag    600
gaaaacgggg actgtggtac gtggagcaac gtgcaaacag agttatgttt caactgcaac    660
gcatgcaaag cgggtgtggt agcgaacata agagagaagt ggaggaatct tcttgttttc    720
aacatttgtc tctcattct cctcataacc gtctattcct gcggttgcg tgctcgtcgt    780
aacaatcgga cggctaggaa aagtgattct gtctga                                816

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<210> SEQ ID NO 19

<211> LENGTH: 271

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 19

```

Met Phe Arg Val Ser Asn Phe Met Val Gly Leu Ala Asn Thr Leu Val
  1           5           10           15
Met Leu Val Gly Ala Ser Ala Ile Gly Tyr Ser Ile Tyr Met Phe Val
           20           25           30
His Gln Gly Val Thr Asp Cys Glu Ser Ala Ile Arg Ile Pro Leu Leu
           35           40           45
Thr Thr Gly Leu Ile Leu Phe Leu Val Ser Leu Leu Gly Val Ile Gly
           50           55           60
Ser Cys Phe Lys Glu Asn Leu Ala Met Val Ser Tyr Leu Ile Ile Leu
           65           70           75           80
Phe Gly Gly Ile Val Ala Leu Met Ile Phe Ser Ile Phe Leu Phe Phe
           85           90           95
Val Thr Asn Lys Gly Ala Gly Arg Val Val Ser Gly Arg Gly Tyr Lys
           100          105          110
Glu Tyr Arg Thr Val Asp Phe Ser Thr Trp Leu Asn Gly Phe Val Gly
           115          120          125
Gly Lys Arg Trp Val Gly Ile Arg Ser Cys Leu Ala Glu Ala Asn Val
           130          135          140
Cys Asp Asp Leu Ser Asp Gly Arg Val Ser Gln Ile Ala Asp Ala Phe
           145          150          155          160
Tyr His Lys Asn Leu Ser Pro Ile Gln Ser Gly Cys Cys Lys Pro Pro
           165          170          175
Ser Asp Cys Asn Phe Glu Phe Arg Asn Ala Thr Phe Trp Ile Pro Pro
           180          185          190
Ser Lys Asn Glu Thr Ala Val Ala Glu Asn Gly Asp Cys Gly Thr Trp
           195          200          205
Ser Asn Val Gln Thr Glu Leu Cys Phe Asn Cys Asn Ala Cys Lys Ala
           210          215          220

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Gln Leu Phe Thr Val Leu Ala Val Ala Arg Leu Lys Thr Pro Asn Ala
 165 170 175
 Leu Asn Ile Thr Ala Ser Leu Met Asp Val Thr Ala Cys Pro Asn Pro
 180 185 190
 Asp Leu Asp Gly Asn Ser Pro Gly Leu Phe Ser Phe Leu Met Gln Gln
 195 200 205
 Leu Phe Leu Leu Phe Ser Arg His Val Gly Gln Gly Gly Gly Met Gly
 210 215 220
 Glu Ile Gly Ile Ser Glu Lys Tyr Leu Cys
 225 230

<210> SEQ ID NO 22
 <211> LENGTH: 795
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 22

atgctccggc taagcaacgc cgccgtaata acaaccaatg caattctcgc attgatcggc 60
 ctgcccgcctc tatctttttc cgtctacgtc tacgttcaag gcccatcaca gtgtcaacgt 120
 ttcgttcaaa accctctcat tgtaactgcy gctctcctct tcttcatctc gtccttaggc 180
 cttatcgctg ctctctacgg tagccacatc atcatcacac tctatctctt cttccttttc 240
 ctctccattc ttctgcttct tgctctctct gtctttatct tcctcgtcac gaatcccacc 300
 gccggaaaag cgttatccgg tagaggaata ggcaatgtca agaccggaga ttatcagaac 360
 tggatcggga accatttctc tcgtgggaag aattgggaag ggatcaccaa atgtttgtct 420
 gattctaggg tttgtaaag gtttggtcca cgtgacattg actttgactc caaacatctc 480
 tctaattgtac agtttggttg ttgtcgacct cccgtagaat gtgggttcga atcaaagaat 540
 gccacgtggt ggacagttcc tgccacagcg actacggcga ttatagggga ttgtaaggca 600
 tggagtaaca cgcagagaca gttatgttac gcgtgcgagt cgtgtaagat tggagtttta 660
 aaagggataa gaaaagatg gaggatactt attgtcgtca atctccttct tatecttctc 720
 gtcgtttttc tttactcgtg tggctgttgc gtgagaaaga acaatcgtgt tccatggaag 780
 cgccggttct tctaa 795

<210> SEQ ID NO 23
 <211> LENGTH: 264
 <212> TYPE: PRT
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 23

Met Leu Arg Leu Ser Asn Ala Ala Val Ile Thr Thr Asn Ala Ile Leu
 1 5 10 15
 Ala Leu Ile Gly Leu Ala Ala Leu Ser Phe Ser Val Tyr Val Tyr Val
 20 25 30
 Gln Gly Pro Ser Gln Cys Gln Arg Phe Val Gln Asn Pro Leu Ile Val
 35 40 45
 Thr Ala Ala Leu Leu Phe Phe Ile Ser Ser Leu Gly Leu Ile Ala Ala
 50 55 60
 Leu Tyr Gly Ser His Ile Ile Ile Thr Leu Tyr Leu Phe Phe Leu Phe
 65 70 75 80
 Leu Ser Ile Leu Leu Leu Leu Val Leu Ser Val Phe Ile Phe Leu Val
 85 90 95
 Thr Asn Pro Thr Ala Gly Lys Ala Leu Ser Gly Arg Gly Ile Gly Asn
 100 105 110

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Val Lys Thr Gly Asp Tyr Gln Asn Trp Ile Gly Asn His Phe Leu Arg
 115 120 125

Gly Lys Asn Trp Glu Gly Ile Thr Lys Cys Leu Ser Asp Ser Arg Val
 130 135 140

Cys Lys Arg Phe Gly Pro Arg Asp Ile Asp Phe Asp Ser Lys His Leu
 145 150 155 160

Ser Asn Val Gln Phe Gly Cys Cys Arg Pro Pro Val Glu Cys Gly Phe
 165 170 175

Glu Ser Lys Asn Ala Thr Trp Trp Thr Val Pro Ala Thr Ala Thr Thr
 180 185 190

Ala Ile Ile Gly Asp Cys Lys Ala Trp Ser Asn Thr Gln Arg Gln Leu
 195 200 205

Cys Tyr Ala Cys Glu Ser Cys Lys Ile Gly Val Leu Lys Gly Ile Arg
 210 215 220

Lys Arg Trp Arg Ile Leu Ile Val Val Asn Leu Leu Leu Ile Leu Leu
 225 230 235 240

Val Val Phe Leu Tyr Ser Cys Gly Cys Cys Val Arg Lys Asn Asn Arg
 245 250 255

Val Pro Trp Lys Arg Arg Phe Phe
 260

<210> SEQ ID NO 24
 <211> LENGTH: 654
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 24

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atgattgatt ttctttgaa gtatcttgcc gtgctcctga tcgttttgat cgcgattcct    60
gtctttaccg tactggcggt cattgtaaca aacaatgggt ctggccatac taaccctggt    120
ttaaggtaca aggagtataa gctgaatgat tacagctcat ggtttctaaa acagcttaac    180
aacaccagta actggataag actaaagagt tgtcttgta aatccgagca atgtcggag    240
ctttccaaga aatacaagac catcaaacag ttgaaatcgg cagaattaac cccgatagaa    300
gctggatggt gtcgaccacc atctgagtgt ggttatcctg cggtgaatgc ttcttactat    360
gacttgagct ttcattcgat aagttctaac aaagattgta agctttacaa gaatttgagg    420
actatcaagt gctacaactg tgattcttgc aaagctggag ttgctcagta catgaaaacc    480
gagtgccgac ttgttgcgat cttcaatgtg gtctgtttg ttgtcttgat aagctctctt    540
cttagcacga gatttgactc tgaacaaagt tttggccttt taaacggttt agtgcaaatt    600
tccaacataa cttttaaga ttgcaaac acaacagtac caaacagtt ttaa                654

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<210> SEQ ID NO 25
 <211> LENGTH: 217
 <212> TYPE: PRT
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 25

Met Ile Asp Phe Pro Leu Lys Tyr Leu Ala Val Leu Leu Ile Val Leu
 1 5 10 15

Ile Ala Ile Leu Val Phe Thr Val Leu Ala Phe Ile Val Thr Asn Asn
 20 25 30

Gly Ser Gly His Thr Asn Pro Gly Leu Arg Tyr Lys Glu Tyr Lys Leu
 35 40 45

Asn Asp Tyr Ser Ser Trp Phe Leu Lys Gln Leu Asn Asn Thr Ser Asn
 50 55 60

-continued

Trp Ile Arg Leu Lys Ser Cys Leu Val Lys Ser Glu Gln Cys Arg Lys
65 70 75 80

Leu Ser Lys Lys Tyr Lys Thr Ile Lys Gln Leu Lys Ser Ala Glu Leu
85 90 95

Thr Pro Ile Glu Ala Gly Cys Cys Arg Pro Pro Ser Glu Cys Gly Tyr
100 105 110

Pro Ala Val Asn Ala Ser Tyr Tyr Asp Leu Ser Phe His Ser Ile Ser
115 120 125

Ser Asn Lys Asp Cys Lys Leu Tyr Lys Asn Leu Arg Thr Ile Lys Cys
130 135 140

Tyr Asn Cys Asp Ser Cys Lys Ala Gly Val Ala Gln Tyr Met Lys Thr
145 150 155 160

Glu Trp Arg Leu Val Ala Ile Phe Asn Val Val Leu Phe Val Val Leu
165 170 175

Ile Ser Ser Leu Leu Ser Thr Arg Phe Asp Ser Glu Gln Ser Phe Gly
180 185 190

Leu Leu Asn Gly Leu Val Gln Ile Ser Asn Ile Thr Phe Lys Asp Cys
195 200 205

Gln Thr Thr Thr Val Pro Lys Gln Phe
210 215

<210> SEQ ID NO 26

<211> LENGTH: 837

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 26

```

atggcgagag ataaagaaga tcaaacaat gagaatcctt caattgtcca gaacatgtca    60
tttcattca acaccatttt cttgatctca agcgcaatct tcctcgtcac agccgctttc    120
tggttcgtag ccgctcatgac attacattac aggaccgatg aatgtaaccg gttcgtcaca    180
actcccggaa tattcataag ctttccattg cttgctatgt ccctcactgg attctacgca    240
gcttacttca aatccgattg tctcttttga atccacttct ttatcttctt cttgtggatg    300
ttcgttgctg tgtctaaagc aatctttgtc atctttctac ataaggagac caatcctaga    360
ttgtttcctg ggaccaagat ttatgagttt aggtacgagg attactcagg atgggttagt    420
agattggtca tcaaagacga tgaatggtat cgtacaagga gatgtcttgt taaggacaat    480
gtttgtaaca ggctaaacca taagatgcca gttcttgagt tttatcagat gaatctaact    540
cctatacagt cgggttggtg caaaccacca ctttcatgtg gattgaatta cgagaaacca    600
aataattgga cagtttcaag atattataac aatttagaag ttgattgcaa gagatggaac    660
aattctgcag atacattatg cttcgtattg gattcatgta aagctgtgat tattgtctgat    720
gtacataata cttcattttc cataacagtt aacattattc atatcatctt tagtctttgt    780
atcgcatga cgggttggtt tgcctgggta aggatccttc gagaaagtca gaaatag      837

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<210> SEQ ID NO 27

<211> LENGTH: 278

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 27

Met Ala Arg Asp Lys Glu Asp Gln Asn Asn Glu Asn Pro Ser Ile Val
1 5 10 15

Gln Asn Met Ser Phe Pro Phe Asn Thr Ile Phe Leu Ile Ser Ser Ala
20 25 30

-continued

Ile Phe Leu Val Thr Ala Ala Phe Trp Phe Val Ala Val Met Thr Leu
 35 40 45

His Tyr Arg Thr Asp Glu Cys Asn Arg Phe Val Thr Thr Pro Gly Ile
 50 55 60

Phe Ile Ser Phe Ser Leu Leu Ala Met Ser Leu Thr Gly Phe Tyr Ala
 65 70 75 80

Ala Tyr Phe Lys Ser Asp Cys Leu Phe Arg Ile His Phe Phe Ile Phe
 85 90 95

Phe Leu Trp Met Phe Val Val Val Ser Lys Ala Ile Phe Val Ile Phe
 100 105 110

Leu His Lys Glu Thr Asn Pro Arg Leu Phe Pro Gly Thr Lys Ile Tyr
 115 120 125

Glu Phe Arg Tyr Glu Asp Tyr Ser Gly Trp Val Ser Arg Leu Val Ile
 130 135 140

Lys Asp Asp Glu Trp Tyr Arg Thr Arg Arg Cys Leu Val Lys Asp Asn
 145 150 155 160

Val Cys Asn Arg Leu Asn His Lys Met Pro Ala Ser Glu Phe Tyr Gln
 165 170 175

Met Asn Leu Thr Pro Ile Gln Ser Gly Cys Cys Lys Pro Pro Leu Ser
 180 185 190

Cys Gly Leu Asn Tyr Glu Lys Pro Asn Asn Trp Thr Val Ser Arg Tyr
 195 200 205

Tyr Asn Asn Leu Glu Val Asp Cys Lys Arg Trp Asn Asn Ser Ala Asp
 210 215 220

Thr Leu Cys Phe Asp Cys Asp Ser Cys Lys Ala Val Ile Ile Ala Asp
 225 230 235 240

Val His Asn Thr Ser Phe Ser Ile Thr Val Asn Ile Ile His Ile Ile
 245 250 255

Phe Ser Leu Cys Ile Gly Met Thr Gly Trp Phe Ala Trp Leu Arg Ile
 260 265 270

Leu Arg Glu Ser Gln Lys
 275

<210> SEQ ID NO 28

<211> LENGTH: 816

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 28

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atggaacat tgatggcact tgtgaacatt ttagccgctg gtgtccttcc gatcttcaact    60
ttcgtcctct cacttacact cctcggctac gcagtggtggc ttctttacat gcgtagctac    120
gactgcgaag atattctcgg tctgccacgt gtccagacgc tagctagtgt cggctcttctc    180
gcggtgtttg ttgtcagcaa cgcagctctg tttttgctggc ggaagtttcc gatgcctgca    240
cttgtggtga tgggtggtgt cttgttgta atgcttttca tcggtttggc gtatgcgga    300
gtaaatagaga tgcaaagccg gcggtttccg ggcacaagga tgtggttcaa gctcaaaatc    360
atggacgatac atgtgacctg gaacaatata aatcgtgtg tctatgataa aggagcttgc    420
aacgacctca tttacggatc tccaaatgaa aaaccttaca atagaagaaa aatgccacca    480
atcaagaatg gatgttgat gccaccagag acatgtaaca tggacgcgat aaacgcgacg    540
ttttgtaca gaagaaaaga cgaaggacca cgcctgctta tgaacctaat gtacggtgat    600
gagatgatgg tgggaaggat tagcgactgt caactatgga ggaacgattg gagcatttta    660
tgctatgatt gtagatcttg taagttcggg ttcataagat cggttaaggag gaaatggtgg    720

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cagctcggta tcttcttgat cgtcatttcc attcttcttc tcatgtctca tctcttgatc 780
ttcttggcta ctttttggga acgattcaag ggtag 816
```

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<210> SEQ ID NO 29
<211> LENGTH: 271
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana
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<400> SEQUENCE: 29
```

```
Met Gly Thr Leu Met Ala Leu Val Asn Ile Leu Ala Ala Gly Val Leu
  1           5           10          15
Pro Ile Phe Thr Phe Val Leu Ser Leu Thr Leu Leu Gly Tyr Ala Val
          20           25           30
Trp Leu Leu Tyr Met Arg Ser Tyr Asp Cys Glu Asp Ile Leu Gly Leu
  35           40           45
Pro Arg Val Gln Thr Leu Ala Ser Val Gly Leu Leu Ala Val Phe Val
  50           55           60
Val Ser Asn Ala Ala Leu Phe Leu Arg Arg Lys Phe Pro Met Pro Ala
  65           70           75           80
Leu Val Val Met Val Val Val Leu Leu Leu Met Leu Phe Ile Gly Leu
  85           90           95
Ala Tyr Ala Gly Val Asn Glu Met Gln Ser Arg Arg Phe Pro Ala Thr
 100          105          110
Arg Met Trp Phe Lys Leu Lys Ile Met Asp Asp His Val Thr Trp Asn
 115          120          125
Asn Ile Lys Ser Cys Val Tyr Asp Lys Gly Ala Cys Asn Asp Leu Ile
 130          135          140
Tyr Gly Ser Pro Asn Glu Lys Pro Tyr Asn Arg Arg Lys Met Pro Pro
 145          150          155          160
Ile Lys Asn Gly Cys Cys Met Pro Pro Glu Thr Cys Asn Met Asp Ala
 165          170          175
Ile Asn Ala Thr Phe Trp Tyr Arg Arg Lys Asp Glu Gly Pro Pro Ser
 180          185          190
Ser Met Asn Leu Met Tyr Gly Asp Glu Met Met Val Gly Arg Ile Ser
 195          200          205
Asp Cys Gln Leu Trp Arg Asn Asp Trp Ser Ile Leu Cys Tyr Asp Cys
 210          215          220
Arg Ser Cys Lys Phe Gly Phe Ile Arg Ser Val Arg Arg Lys Trp Trp
 225          230          235          240
Gln Leu Gly Ile Phe Leu Ile Val Ile Ser Ile Leu Leu Leu Met Ser
 245          250          255
His Leu Leu Ile Phe Leu Ala Thr Phe Trp Glu Arg Phe Lys Gly
 260          265          270
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<210> SEQ ID NO 30
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:vector
      pSKI015
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<400> SEQUENCE: 30
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gcggcagcgg cggcaggata tatt
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<210> SEQ ID NO 31
 <211> LENGTH: 24
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer
 <400> SEQUENCE: 31
 tgctttcgcc tataaatacg acgg 24

<210> SEQ ID NO 32
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer
 <400> SEQUENCE: 32
 cgctgaggac atctacattt ttg 23

<210> SEQ ID NO 33
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer
 <400> SEQUENCE: 33
 tcccggacat gaagccattt ac 22

<210> SEQ ID NO 34
 <211> LENGTH: 16
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: modified_base
 <222> LOCATION: 1 and 11
 <223> OTHER INFORMATION: n represents a,g,c or t
 <220> FEATURE:
 <221> NAME/KEY: modified_base
 <222> LOCATION: 7
 <223> OTHER INFORMATION: s represents g or c
 <220> FEATURE:
 <221> NAME/KEY: modified_base
 <222> LOCATION: 8 and 13
 <223> OTHER INFORMATION: w represents a or t
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer
 <400> SEQUENCE: 34
 ngtcgaswga nawgaa 16

<210> SEQ ID NO 35
 <211> LENGTH: 278
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: modified_base
 <222> LOCATION: 13, 35, 73, 108, 156, 190, 198 and 201
 <223> OTHER INFORMATION: n represents a,g,c or t
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:PCR product
 from Arabidopsis thaliana
 <400> SEQUENCE: 35
 tgtggcaaac tcngagtagg aatggagaat ccaancgttt gggcttttct caaggaagaa 60
 agtgacgact gncattgta ttgctccgaa taaacacatc catgctgnta aagagagggt 120
 atctggatag taggcagaga ttggaacctt gggttntggt gaaacaaaac tcacatttct 180

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 tgtaagacan gaaacatnca ncaacaaaaa gtttagactt ttgatttatt taatgaagtt 240

acctgaagta taagccaaaa ggaccaacaa agagtgct 278

<210> SEQ ID NO 36
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer

<400> SEQUENCE: 36

cttcttcaat catcaccatg 20

<210> SEQ ID NO 37
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer

<400> SEQUENCE: 37

tagcttgaac cggcgcaaat 20

<210> SEQ ID NO 38
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer

<400> SEQUENCE: 38

gtacgtttta gtaacagtct 20

<210> SEQ ID NO 39
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer

<400> SEQUENCE: 39

gattagcagt gactaactcc 20

<210> SEQ ID NO 40
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer

<400> SEQUENCE: 40

agcttgata ttaaccgtga ct 22

<210> SEQ ID NO 41
 <211> LENGTH: 25
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:primer

<400> SEQUENCE: 41

ctgcagggtg atgattgaag aagat 25

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What is claimed is:

1. A method for screening for a transgenic plant, comprising
 - introducing a recombinant vector into a plant, wherein the recombinant vector comprises a gene encoding a protein of the following (a) or (b):
 - (a) a protein comprising the amino acid sequence represented by SEQ ID NO: 2;
 - (b) a protein consisting of an amino acid sequence having a substitution, deletion or addition of one to ten amino acids relative to the amino acid sequence represented by SEQ ID NO: 2 and capable of imparting paraquat resistance;
 and wherein the recombinant vector further comprises a foreign gene or a foreign DNA fragment; and
 - screening for a transgenic plant on the basis of paraquat resistance as an indicator.
2. The method of claim 1, wherein the screening for a transgenic plant comprises growing the plant in a paraquat-containing medium.
3. The method of claim 2, wherein the paraquat in the paraquat-containing medium is present at a concentration of between 0.1 and 3.0 μM .
4. The method of claim 3, wherein the paraquat in the paraquat-containing medium is present at a concentration of 3.0 μM .
5. A method for imparting paraquat resistance to a plant, comprising
 - introducing a recombinant vector into a plant, wherein the recombinant vector comprises a gene encoding a protein of the following (a) or (b):
 - (a) a protein comprising the amino acid sequence represented by SEQ ID NO: 2;
 - (b) a protein consisting of an amino acid sequence having a substitution, deletion or addition of one to ten amino acids relative to the amino acid sequence represented by SEQ ID NO: 2 and capable of imparting paraquat resistance;
 imparting paraquat resistance; and
 - confirming paraquat resistance.

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6. The method of claim 5, wherein paraquat resistance is confirmed by determining that the plant is capable of growing in the presence of paraquat.
7. The method of claim 6, wherein the paraquat is present in a culture medium.
8. The method of claim 7, wherein the paraquat is present at a concentration of between 0.1 and 3.0 μM .
9. The method of claim 8, wherein the paraquat is present at a concentration of 3.0 μM .
10. A method for screening for a transgenic plant, comprising
 - introducing a recombinant vector into a plant, wherein the recombinant vector comprises a gene encoding a protein of the following (a) or (b):
 - (a) a protein comprising the amino acid sequence represented by SEQ ID NO: 2;
 - (b) a protein consisting of an amino acid sequence having a substitution, deletion or addition of one to ten amino acids relative to the amino acid sequence represented by SEQ ID NO: 2 and capable of imparting paraquat resistance; and
 screening for a transgenic plant on the basis of paraquat resistance as an indicator.
 11. The method of claim 10, wherein the screening for a transgenic plant comprises growing the plant in a paraquat-containing medium.
 12. The method of claim 11, wherein the paraquat in the paraquat-containing medium is present at a concentration of between 0.1 and 3.0 μM .
 13. The method of claim 12, wherein the paraquat in the paraquat-containing medium is present at a concentration of 3.0 μM .
 14. The method of claim 1, 5, or 10, wherein the plant is selected from Poaceae, Brassicaceae, Solanaceae, and Leguminosae.
 15. The method of claim 14, wherein the plant is a Brassicaceae, and the Brassicaceae is *Arabidopsis thaliana*.

* * * * *